

B39



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/12, C07K 14/705, G01N 33/68		A2	(11) International Publication Number: WO 00/08149
			(43) International Publication Date: 17 February 2000 (17.02.00)
(21) International Application Number: PCT/IB99/01445 (22) International Filing Date: 5 August 1999 (05.08.99) (30) Priority Data: 60/095,408 5 August 1998 (05.08.98) US (71) Applicant: CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE - CNRS [FR/FR]; 3, rue Michel Ange, F-75794 Paris Cedex 16 (FR). (72) Inventors: WALDMANN, Rainer; Chemin de Chense, F-83600 Les Adrets de l'Esterel (FR). BASSILANA, Frédéric; 15, rue Deldille, F-06000 Nice (FR). LAZDUNSKI, Michel; 21, avenue Colombo, F-06000 Nice (FR). DE WEILLE, Jan, R.; Centre National de la Recherche Scientifique - CNRS, 3, rue Michel Ange, F-75794 Paris Cedex 16 (FR). (74) Agents: BREESE, Pierre et al.; Breese-Majerowicz, 3, avenue de l'Opéra, F-75001 Paris (FR).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>	
(54) Title: HUMAN NEURONAL ACID-SENSITIVE CATIONIC CHANNELS, ITS CLONING AND APPLICATIONS			
(57) Abstract <p>Non-inactivating or slowly inactivating proton-gated cation channels are thought to play an important role in the perception of pain that accompanies tissue acidosis. We have identified a human proton-gated cation channel subunit that has biphasic desensitisation kinetics with both a rapidly inactivating Na⁺-selective and a sustained component. The protein shares 84 % sequence identity with the proton-gated cation channel rASIC3 (rDRASIC) from rat sensory neurones. The biphasic desensitisation kinetics and the sequence homology suggest that this clone (hASIC3) is the human orthologue of rASIC3 (rDRASIC). While rASIC3 (rDRASIC) requires very acidic pH (< pH 4.5) for activation of the sustained current, the non-inactivating hASIC3 current starts to be activated when the pH decreases to below pH 6. hASIC3 is an acid sensor and might play an important role in the detection of lasting pH changes in human. We localized the hASIC3 gene to the human chromosome 7q35, 6.4 cRad telomeric from the microsatellite AFMA082XC9.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BP	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LJ	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

HUMAN NEURONAL ACID-SENSITIVE CATIONIC CHANNELS, ITS CLONING AND APPLICATIONS

BACKGROUND OF THE INVENTION5 *Cross-Reference To Related Patent Applications*

This patent application benefits of and claims the benefit of provisional patent application serial no. 60/095,408, *Identification, Functional Expression and Chromosomal Localisation Of Sustained Human-Proton-Gated Cation Channel*, filed on August 5, 1998, and application serial no. 09/129,758,
10 *Mammal Neuronal Acid Sensing Cationic Channel, Cloning and Application Thereof* filed on August 5, 1998. The said U.S. applications being incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to new families of mammalian, notably
15 human and rat, acidity-sensitive ionic channels. More particularly, the invention relates to the identification and molecular characterization in humans and rats of a new family of proton-activated cationic channels, collectively referred to below as ASIC polypeptides, for Acid Sensing Ionic Channel.

20 The ASIC channels constitute the first members of a group of cationic channels belonging to the family of amiloride-sensitive degenerine sodium channels [6, 11-14], which are activated temporarily by extracellular acidification.

Sensitivity to acid is associated with both nociception [1] and the transduction of taste [2]. The stimulation of sensory neurons by acids is of great importance because acidity accompanies numerous painful inflammatory and ischemic situations. The pain caused by acids is thought to

5 be mediated by the cationic channels present at the level of the sensory neurons which are activated by protons [3-5]. The biophysical and pharmacological properties of the ASIC channels of the invention are similar to those of the proton-activated cationic channels described in the sensory neurons [3, 15, 16]. However, as will be seen in the description below, to

10 date there has been no report of ligand-activated ionic channels simpler than the ASIC channels.

Summary of the Invention

The invention also relates to hybrid cationic channels constituted by the combination of a first protein comprising a proton-activated ionic channel

15 according to the invention with a second proton-activated ionic channel.

The present invention has as its object a nucleic acid molecule coding for a protein constituting a neuronal neuronal cationic channels that is sensitive to amiloride and activated by protons.

The invention also relates to a vector comprising at least one of the

20 preceding nucleic acid molecules, advantageously combined with suitable control sequences, as well as a procedure for production or expression in a cell host of a protein constituting an ionic channel according to the Invention.

The invention also relates to the transformed cells expressing ASIC cation channels and/or their derivatives obtained according to the preceding methods.

The present invention also relates to application of the ASIC channel
5 for studying pathological modifications that may lead to neuronal degenerations. The invention this also relates to the pharmaceutical preparations comprising as an active ingredient, at least one of these proteins of the invention.

Other characteristics and advantages of the invention will be seen in
10 the description below related to research activities that led to the demonstration and the characterization of the ASIC channel.

This invention can be further understood with reference to the Figures, discussed next and in the Examples.

15

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 represents the alignment of the sequences of the rat ASIC proteins (at top) and human ASIC proteins (at bottom) of sequences SEQ ID NO: 1 and SEQ ID NO: 2.

Figure 2 represents a comparison of the protein sequence of the
20 rASIC1A channel with the sequence of other ionic channels:

Figure 3 represents the phylogenetic tree of the proteins of the subunits α NaCh, β NaCh, γ NaCh, δ NaCh of the amiloride-sensitive sodium channel and of the degenerines MEC-4, MEC-10 and DEG-1 of *C. elegans*.

Figure 4 represents the topology proposed for this latter family of ionic channels [30].

Figure 5 shows the biophysical properties of the proton-activated rASIC1A channel.

5 **Figure 6** shows the effect of Ca^{2+} and of amiloride on the rASIC1A current.

Figure 7 shows the tissue distribution of ASIC1A channel mRNA.

Figure 8 shows the *in situ* hybridization.

Figure 9 shows the alignment of the deduced protein sequences of
10 hASIC3 and rASIC3.

Figure 10 shows the pH dependence and pharmacology of hASIC3.

Figure 11 shows the selectivity and single channel properties of hASIC3.

Figure 12 shows the human chromosomal localization of the hASIC3
15 gene.

IDENTIFICATION OF THE AMINO ACID AND DNA SEQUENCES

SEQ ID NO: 1 represents the sequence of 526 amino acids of the protein of the rASIC1A channel deduced from the cDNA sequence of the rat.

SEQ ID NO: 2 represents the partial sequence of 514 amino acids of
20 the protein of the hASIC1A channel deduced from the partial sequence of human cDNA.

SEQ ID NO: 3 represents the sequence of 512 amino acids of the protein of the hASIC2A channel deduced from the sequence of human cDNA.

SEQ ID NO: 4 represents the sequence of 559 amino acids of the protein of the rASIC1B channel as well as the sequence of a DNA molecule comprising the sequence coding for that protein.

SEQ ID NO: 5 represents the sequence of 533 amino acids of the protein of the rASIC3 channel and the sequence of DNA coding for that protein.

SEQ ID NO: 6 represents the sequence of 563 amino acids of the protein of the rASIC2B channel as well as the sequence of a DNA molecule comprising the sequence coding for that protein.

10 SEQ ID NO: 7 represents the sequence of 533 amino acids of the protein of the hASIC3 channel as well as the sequence of a DNA molecule comprising the sequence coding for that protein.

DETAILED DESCRIPTION OF THE INVENTION

15 The present invention has as its object and rat proteins constituting neuronal cationic channels that are sensitive to amiloride and which are activated by protons. The invention relates to proteins constituting the ASIC family of cation channels, or functionality equivalent derivatives of these proteins.

20 Such derivatives are those polypeptides whose sequence includes a modification and/or a suppression and/or an addition of one or more amino acid residues, as long as this modification, suppression, and/or addition does not alter the functional and structural properties of the ASIC channel, princi-

pally its activation by protons. Indeed, three different ASIC polypeptides, ASIC1, ASIC2 and ASIC3, in both rat and human, are described herein. In addition, the transcripts encoding ASIC1 and ASIC2 are alternatively spliced, which generates additional functional derivatives of the ASIC1 and ASIC2

5 proteins (ASIC1A and 1B, ASIC2A and ASIC2B, respectively). Other functional derivatives of the ASIC proteins and/or other forms of the ASIC polypeptides generated by alternative splicing of the ASIC mRNA transcripts are considered to be within the scope of the present invention. Such proteins and their functional derivatives can be analyzed by an expert in the field

10 using the techniques described in the Examples included herein, which make it possible to demonstrate the biophysical and pharmacological properties of the ASIC channels.

Further examples of functional derivatives of the ASIC channels are as follows: The human and rat ASIC1A proteins (hASIC1A and rASIC1A, SEQ ID

15 Nos. 1 and 2, respectively) are considered to be functionally equivalent. The amino acid sequences of these two proteins are highly homologous, but they are not identical. Thus, substitutions can readily be introduced within the primary sequence of ASIC proteins without influencing their basic functional characteristics.

20 Another example of such a functionally equivalent derivative is the protein constituting a cationic channel previously designated MDEG [14] or BNaCl [20], designated herein as rASIC2A. The amino acid sequence of rASIC2A is represented in the annexed list of sequences under number SEQ

ID NO: 3. rASIC2A has been described as a mammalian cationic channel which is sensitive to amiloride and which is activated in *C. elegans* by mutations that result in neurodegeneration. The rASIC2A channel is a structurally similar to the ASIC1A channel, exhibiting approximately 67% homology in their amino acid sequences. Cation transport by both polypeptides is sensitive to amiloride and regulated by acid. However, the electrophysiological properties of these two channels are different because they are not activated by the same pH changes. Thus, the range of sensitivity of rASIC2A ($EC_{50} = 4.05$) is different from that of ASIC1A ($EC_{50} = 6.2$). Other functionally equivalent proteins that may exhibit different electrophysiological properties are also considered to be within the scope of the invention.

It has been shown that the rASIC2A channel is activated by the same mutations as those causing neuronal degeneration in *C. elegans*. Thus, like the hyperactive mutants of *C. elegans*, the active mutants of rASIC2A are responsible for cell death. This indicates that the acquisition of function by this neuronal ionic channel could be associated with various forms of neuronal degeneration in mammals, notably of rodents and humans. However, no normal physiological function of rASIC2A was known until the demonstration of its activation by protons in accordance with the cationic channels of the present invention.

Other examples of proteins constituting a neuronal cationic channel that are sensitive to amiloride and activated by protons according to the invention are presented below

- A channel designated ASIC1B, whose sequence of 559 amino acids is represented in the annexed list of sequences under number SEQ ID NO: 4. ASIC1B is a splicing variant of the ASIC1A channel, cloned from the rat brain by degenerated PCR. The first 185 amino acids are replaced by a new sequence of 218 amino acids which is underlined in SEQ ID NO: 4.

- A channel designated rASIC2B. rASIC2B is a splicing variant of rASIC2A and is represented by SEQ ID No. 6.

- A channel designated rASIC3, whose sequence of 533 amino acids is represented in the list of sequences under number SEQ ID NO: 5. rASIC3 was cloned from sensory neurons from the rat using a partial sequence from the data banks (Expressed Sequence Tag with accession number W62694). The properties of rASIC3 are as follows:

- a) It is expressed in the sensory neurons but not in the brain.
- b) Its expression in *Xenopus* oocytes or in mammalian cells allows recording of a proton-activated sodium current which presents two components: a component activating and inactivating itself rapidly, and a component activating itself more slowly and not inactivating itself. The two components are selective for Na⁺. A proton-activated cationic channel that does not inactivate itself was implicated in the prolonged sensation of pain caused by acidosis.

- A channel designated hASIC3, which is represented by SEQ ID No. 7. This protein is a novel human proton-gated cation channel subunit that has biphasic desensitisation kinetics, with both a rapidly inactivating Na⁺-

selective and a sustained component. The protein shares 84% sequence identity with the proton-gated cation channel rASIC3 from rat sensory neurons.

The invention also relates to hybrid cationic channels, or channels
5 constituted by the combination of a first protein comprising a proton-activated ionic channel according to the invention with a second protein comprising a proton-activated ionic channel. Advantageously, the said second protein is also a protein comprising a proton-activated ionic channel according to the invention. An example of such a combination is illustrated by the combination
10 of the ASIC1A, ASIC2A or ASIC3 channel with the ASIC2A channel. Such hybrid channels exhibit a third range of pH sensitivity (e.g., with ASIC: $EC_{50} = 4.8$). Another example of such a hybrid channel is the combination of the ASIC1A, ASIC1B, ASIC2A or ASIC3 channels with the the ASIC2B channel.

ASIC2B is a channel that was cloned from the rat brain using a partial
15 mouse sequence accessible in the data banks (Expressed Sequence Tag with accession number W50528) and whose sequence of 563 amino acids is represented in the annexed list of sequences under number SEQ ID NO: 6. ASIC2B is a splicing variant of ASIC2A. The first 185 amino acids are replaced by a new sequence of 236 amino acids which is underlined in SEQ ID
20 NO: 6. ASIC2B is expressed in the brain and in the sensory neurons of the dorsal root ganglia.

ASIC2B expressed alone in *Xenopus* oocytes or in mammalian cells does not form a proton-activated cationic channel. However, it can combine

with ASIC2A or ASIC3 to form proton-activated heteromultimeric channels with modified properties. The activation pH of the channel formed after the co-expression of ASIC2A and ASIC2B differs from the channel formed by ASIC2A alone. After expression of ASIC2A and ASIC2B in COS cells, the
5 current has not reached its maximum value at pH 3 whereas the current induced by ASIC2A alone is saturated at a pH between 4.5 and 4.0. In addition, the inactivation kinetics and the ionic selectivity of the channel formed after the co-expression of ASIC2A and ASIC2B are clearly different from those of ASIC2A alone. A current appears which inactivates itself slowly
10 and is barely selective for Na⁺ and K⁺.

In another example, the sodium current obtained after expression of ASIC3 becomes non-selective (it does not differentiate between sodium and potassium) when ASIC2B is co-expressed with ASIC3. This new property is similar to that of the proton-activated cationic channel which is implicated in
15 the prolonged sensation of pain caused by acidosis. It is very probable that ASIC3 and ASIC2B are part of this channel.

The amino acid sequence homologies of the proteins constituting the ASIC1A, ASIC1B channels cited according to the invention are presented in Table 1 below.

20

Table 1

Channel	ASIC 1B	ASIC 1A	ASIC2B	ASIC2A	ASIC3
ASIC1B	100	80	56	61	52

ASIC1A		100	59	68	53
ASIC2B			100	78	48
ASIC2A				100	51
ASIC3					100

5

Polyclonal or monoclonal antibodies directed against at least one protein constituting an ion channel of the invention and/or against a hybrid channel as described above can be prepared by the classic methods described in the literature. The antibodies are useful for investigating the presence of the ionic channels of the invention in various human and animal tissues, and may also be used to inhibit or activate an ASIC channel and/or its derivatives *in vivo*. Such an application may be useful for the treatment of diseases arising from defective ASIC cation transport.

The present invention also has as its object a nucleic acid molecule coding for a protein constituting a neuronal cationic channel that is sensitive to amiloride and activated by protons. More particularly, the invention relates to a nucleic acid molecule comprising at least one sequence coding for a protein constituting the ASIC1A, ASIC1B, ASIC2A, ASIC2B, or ASIC3 cation channels from human or rat.

The invention also relates to a vector comprising at least one of the preceding nucleic acid molecules, advantageously combined with suitable control sequences, as well as a procedure for production or expression in a cell host of a protein constituting an ionic channel according to the invention. The preparation of these vectors as well as the production or expression of the

channels of the invention in a competent host cell can be accomplished by established methods known to experts in the field.

For example, the expression and production of a protein constituting a cationic channel according to the invention can be accomplished by:

- 5 - transferring a nucleic acid molecule of the invention or a vector containing said molecule into a competent host cell,
- culturing said host cell host under conditions allowing expression of the ionic channels of the invention.
- isolating the proteins constituting the ionic channels of the invention.

10 The host cell employed in the preceding methods can be selected from among the prokaryotes or the eukaryotes and notably from among the bacteria, yeasts or cells of mammals, plants or insects.

The vector used is selected in relation to the host to which it will be transferred; any vector such as a plasmid can be used.

15 The invention also relates to the transformed cells expressing ASIC cation channels and/or their derivatives obtained according to the preceding methods. These cells are useful for screening to identify substances that are capable of modulating cation transport by these polypeptides and hence, the perception of acidity with regard to both nociception and taste transduction. This
20 screening is implemented by bringing variable quantities of a substance to be tested into contact with cells expressing the ASIC channels and determining the effects of said substance on the currents of said cation channels. These screenings allow for the identification of new drugs that are useful in the

treatment or prevention of pain. They also enable the identification and investigation of agents that modulate acid taste. In addition, these methods are useful for identifying substances that block, or can inhibit neurodegeneration induced by hyperexpression of these channels. The substances which are
5 isolated and detected by means of the methods above are also part of the invention. The ASIC channels clearly have ionic selectivity properties, notably with regard to their selective permeability by sodium, potassium and calcium, which endows them with excitotoxic properties when hyperstimulated.

A protein constituting an ASIC neuronal ionic channel can also be
10 useful for developing drugs intended for the treatment or prevention of pathologies entailing the painful perception of acidity which intervenes in inflammatory diseases, ischemias and a certain number of tumors. The invention thus also relates to pharmaceutical compositions comprising as active ingredients, at least one protein constituting an ionic channel according to the
15 invention.

A nucleic acid molecule coding for a protein constituting an ASIC channel or a derivative thereof, or a vector comprising this nucleic acid molecule or a cell expressing ASIC channels are also useful for the preparation of transgenic animals. These can be animals superexpressing said channels, but also "knock-
20 out" animals, i.e., animals deficient in the expression of these channels or of the cation transport activity of the ASIC channels. These transgenic animals are prepared by methods known to the expert in the field, and enable the

development of live models for studying animal pathologies associated with ASIC channels.

The nucleic acid molecules of the invention or the cells transformed by said molecule can thus be used for genetic therapy to compensate for a deficiency in the ASIC channels at the level of one or more tissues of a patient. The invention thus relates also to a drug comprising nucleic acid molecules of the invention or cells transformed by said nucleic acid molecules for the treatment of pathology involving the ASIC channels or their derivatives.

In addition to the property of being activated by protons and the resultant applications described above relating to the perception of acidity, the ASIC channels, and particularly ASIC channels that have genetic mutations, may be involved in some neurodegenerative processes. The death of certain neurons is characteristic of many types of neuronal degenerative disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis and cerebellar ataxia. Studies of such neurodegenerative processes have identified only a few deficient genes that may be responsible for or associated with these diseases. It is likely that many more important genes remain to be identified. The primitive neural network of the nematode *C. elegans* constitutes a good model of neuronal development and death. The hereditary degeneration in *C. elegans* can be due to mutations of the genes *deg-1*, *mec-4* and *mec-10*. These genes exhibit homology with the subunits of amiloride-sensitive sodium channels. In addition, the functional expression of the *mec-4* chimeras of the epithelial sodium channel, suggest that these genes

are ionic channels whose acquisition of function is the cause of neuronal degeneration.

The present invention thus also relates to application of the ASIC channel for studying these pathological modifications that may lead to neuronal degenerations. The techniques employed for these applications, for example for drug screening, are similar to those described above for the investigation of taste-modulating agents and analgesic agents.

In addition, a protein constituting an ASIC neuronal ionic channel, an agonist or an antagonist of said protein, can also be used for the fabrication of drugs intended for the treatment or prevention of pathologies involving cerebral neuronal degeneration. The invention thus also relates to the pharmaceutical preparations comprising as an active ingredient, at least one of these proteins of the invention, possibly combined with a physiologically acceptable vehicle.

More specifically, the invention relates to a chemical or biological substance that is capable of modifying the currents of an ionic channel and/or a hybrid channel according to the invention for the preparation of a drug capable of modulating the perception of acidity with regard to nociception as well as taste transduction in a human or animal subject.

Other characteristics and advantages of the invention will be seen in the description below related to research activities that led to the demonstration and the characterization of the ASIC channel, and in which reference will be made to the annexed sequences and drawings in which:

Detailed Description of the Figures

Figure 1 represents the alignment of the sequences of the rat ASIC proteins (at top) and human ASIC proteins (at bottom) of sequences SEQ ID NO: 1 and SEQ ID NO: 2. Comparison of these sequences shows the absence of 14 amino acids at the beginning of the human coding phase compared to that of the rat.

Figure 2 represents a comparison of the protein sequence of the rASIC1A channel with the sequence of other ionic channels:

- ASIC2A (MDEG) [14], a mammalian cationic channel that is activated by the mutations responsible for neuro-degenerations with the degenerines of *C. elegans*.

- FaNaCh [10], a peptide of a sodium channel of *Helix aspersa* that is activated by FMRFamide.

- The degenerine MEC-4 [12] of *C. elegans*.

In this figure, the residues that are identical or similar to those of ASIC are printed respectively in white on a black background and in black on a gray background. The supposed transmembranal regions (MI, MII) of rASIC1A are marked by black bars.

Figure 3 represents the phylogenetic tree of the proteins of the subunits α NaCh, β NaCh, γ NaCh, δ NaCh of the amiloride-sensitive sodium channel and of the degenerines MEC-4, MEC-10 and DEG-1 of *C. elegans*.

Figure 4 represents the topology proposed for this latter family of ionic channels [30].

Figure 5 shows the biophysical properties of the proton-activated rASIC1A channel.

a) the macroscopic inflowing currents recorded at -70 mV after rapid pH changes from pH 7.4 to pH 6.

5 b) the dose-response curve of the extracellular pH. The initial pH was 7.4 and the points represent the mean values from 6 tests. The insert in this Figure shows the typical responses at -70 mV.

c) the Q-V relations of the outside-out patch with 140 mM of Na⁺ (■) or of Li⁺ (●) in the bath solution. Q is the charge transported during the acid pH
10 transition. The insert in this figure shows the typical responses in a medium containing Na⁺.

d) the currents activated by the H⁺ protons recorded at various potentials in an outside-out patch in a medium containing Na⁺.

e) the mean i-V relations measured from the outside-out patch with 140
15 mM of Na⁺ (■), 140 mM of Li⁺ (●) or 1.8 mM of Ca²⁺ (▲), as majority permeable ions in the external solutions; the inversion potentials were respectively 65 mV, 58 mV and -34 mV.

f) the proton current through the rASIC1A channel. The relations between the current peak and the voltage were measured from an outside-out patch in
20 a solution of free Na⁺, free Ca²⁺ with pipettes containing a solution of free K⁺, at pH 4 (●) and at pH 3 (■), with (▲) representing the results obtained under the same conditions as (■) but with KCl in the pipette. The insert in this figure shows the typical responses under (▲) conditions.

Figure 6 shows the effect of Ca^{2+} and of amiloride on the rASIC1A current.

a) the currents activated by the H^+ protons recorded at various mem-
branal potentials from an outside-out patch with 1.8 mM of Ca^{2+} in a solution of
5 free Na^+ ; the currents were inverted at -35 mV.

b) the mean Q-V relations from an outside-out patch recorded in solutions
of free Na^+ containing 1.8 mM of Ca^{2+} (○, inversion potential -34 mV) or 0.1 mM
of Ca^{2+} (●, inversion potential -80 mV).

c) the effect of the external Ca^{2+} on the macroscopic peak of inflowing
10 current recorded at -70 mV and activated by a rapid pH change from pH 7.4 to
pH 6. The insert in this Figure shows the typical responses. The points repre-
sent means values \pm se of 5 oocytes.

d) the effect of amiloride on the currents activated by the H^+ protons
recorded at 0 mV from an outside-out patch.

15 e) the inhibition of the macroscopic current (induced by a pH change from
pH 7.4 to pH 6) at -70 mV by amiloride and derivatives. The points represent
the means values \pm se of 5 oocytes.

Figure 7 shows the tissue distribution of ASIC1A channel mRNA.

a) Northern blot analysis of the mRNA expression of the hASIC1A chan-
20 nel in human tissues.

b) In b: RT-PCR analysis of the mRNA expression of the rASIC1A
channel in the rat brain and in the dorsal root ganglion (DRG). (+), (-) represent
respectively the samples with or without reverse transcriptase. The agarose gel

sections were developed in 1% ethidium bromide. The arrows indicate the discounted size (657 pb) of the PCR product.

Figure 8 shows the *in situ* hybridization.

a,b) hybridization of 6µm sections of a dorsal root ganglion from a 3-year-old rat with the E probe marked with digoxigenin. In a: a low-lighting microphotograph (enlargement 30X). In b: a high-resolution image (enlargement 80X) of "a". One can see the intense marking of the small-diameter neurons (arrows). Similar results were also obtained with probes A, C and D.

c) the distribution of the rASIC1A channel mRNA in the brain of an adult rat analyzed by *in situ* hybridization with antisense oligonucleotide C. Identical results were obtained with oligonucleotide B. The colors indicate abundance (red: high expression; blue: not detectable). The abbreviations used in the Figure are as follows: Cer = cerebellum; Hip = hippocampus; OB = olfactory bulb; Cx = cortex.

Figure 9 shows the alignment of the deduced protein sequences of hASIC3 and rASIC3. Amino acids that are identical or similar in both sequences are printed white on black or black on grey background respectively. The two putative hydrophobic transmembrane domains are labelled with boxes. Sequences were aligned with the pileup program (Genetic Computer Group, Wisconsin).

Figure 10 shows the pH dependence and pharmacology of hASIC3. Proton-induced membrane currents were recorded from hASIC3-transfected COS cells using the whole-cell-suction-pipette technique.

a) pH dependence of the hASIC3 current. H^+ -gated currents were induced by decreasing the extracellular pH rapidly from pH 7.3 to the pH values indicated. The pH required for half maximal activation was pH 6.2 for the transient current and pH 4.3 for the sustained current.

5 b) H^+ induced hASIC3 currents depend on the resting pH. The extracellular pH was decreased rapidly from the indicated resting pH to pH 4. The currents in A and B are shown as the fraction of the saturation level of the Boltzmann fit. c) inhibition of hASIC3 by the diuretics amiloride and triamterene. In the dose-response curve for amiloride ($K_{0.5}=15.9 \mu M$), currents are expressed
10 as fraction of the mean current in the absence of drug. Data points (o, transient current; I sustained current) represent the average $\pm SEM$ of at least 5 experiments. Macroscopic currents were recorded from cells clamped at -60 mV using the whole cell suction-pipette technique.

Figure 11 shows the selectivity and single channel properties of hASIC3.

15 a) voltage dependence of the transient and sustained whole cell current. The transient current reverses at 37.6mV, the sustained current reverses at 10.1mV.

b) the voltage dependence of the unitary currents of spontaneously active channels at pH7.3 or of channels activated by a step to pH4. Slope
20 conductance between -10 and +40mV for both conditions is $15.0 \pm 0.6 pS$. $V_{rev} = 30.2 mV$. The Na^+ equilibrium potential is at 40.1mV. Examples of spontaneous channel activity at a resting pH of 7.3 (c) or activity evoked by a drop to pH4 (d). The channel-activity recorded at pH7.3 was inhibited by 100 μM

amiloride (c). Single channel currents were recorded at -60mV from outside-out membrane patches excised from hASIC transfected COS cells.

Figure 12 shows the human chromosomal localization of the hASIC3 gene. The human ASIC3 gene is localized 6.4 cRad telomeric to the framework marker AFMA082XC9 on chromosome 7 (lod score > 21). The position of hASIC3 relative to several microsatellites is shown in the right part of the Figure. The relative positions of the markers and their distances (in cRad) are the output of the RHMAPPER program. The microsatellites D7S676 and D7S642 are localized on band q35 of chromosome 7 (data from <http://www.ncbi.nlm.nih.gov>).
The cytogenic localization of those two markers is indicated with dashed lines.

Cloning the ASIC Channel

The conserved sequences of the family of ASIC ionic channels were used to prepare the following PCR primer sequences:

TTYCCIGCIRTACIITNTGYAAY, and CAIARICCIAITGNCCNCCDAWRTC.

A bank of rat brain cDNA (Stratagene #936515) was hybridized with the PCR product of 1 kB of rat brain and the partial clones were isolated. The fifth extremity of the cDNA (202 bp) was isolated by PCR after ligation adapted to the double-strand cDNA.

20 *Electrophysiology*

0.25 ng of cRNA was injected into the *Xenopus laevis* oocytes and the recording microelectrodes for the imposed voltage and for the patch-clamp were installed two days after the injection. The bath solutions for the outside-

out patch recordings and the pipettes for the outside-out patch and total cells recordings contained: 140 mM KCl (or NMDG), 2 mM $MgCl_2$, 5 mM EGTA, 10 mM Hepes, pH 7.4 (with KOH). The pipettes for the outside-out patch recordings and the bath solutions for the outside-out patch and total cells recordings contained: 140 mM NaCl (or LiCl or NMDGCl), 2 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM Hepes, pH 7.4 (adjusted with HCl, NaOH, LiOH or TMAOH). The rapid pH changes from the initial pH were obtained by perfusion with a bath solution adjusted to the pH indicated in the Figures. The intracellular acidification of the oocytes was implemented by injecting 50 ml of the internal solution at pH 2 or by perfusion and withdrawal of a bath medium containing 20 mM NH_4Cl . None of the recorded currents was contaminated by the Ca^{2+} current sensitive to the Cl^- of the *Xenopus* oocyte. The data were sampled at 2 kHz and filtered at 500 Hz for the analysis. (Logiciel Biopatch).

15 *Northern blot analysis, RT-PCR and in-situ hybridization*

The Northern blot kit was obtained from Clontech Co. (Palo Alto, CA) and contained circa 2 μg of poly(A⁺) RNA per line. The blot was hybridized with a fragment of the partial human clone (corresponding to bases 270 to 764 of the rat clone) marked with ^{32}P at 65°C in 6xSSC. For the RT-PCR analysis, 5 μg of rat brain total RNA and 3 μg of dorsal root ganglion were reverse transcribed and 1/30 of the sample was amplified by 30 PCR cycles with the following sequence primers:

ATTGCTCTTCCCATCTCTAT, and TTCAAGGCCCATACCTAAGT.

The negative controls were treated in an identical manner with the exception of the reverse transcriptase which was not added. The antisense oligonucleotides corresponding to base 70 to 114 (A), 215 to 248 (B), 1821 to 1859 (C), 1896 to 1940 (D) and the double-strand DNA corresponding to
5 base 1685 to 2672 were used for the *in-situ* hybridizations. The sections of adult rat brain were hybridized with oligonucleotides B or C the ends of which were marked with ³²P for one night at 37°C in 50% formamide, 2xSSC, then washed at ambient temperature in 1xSSC. The signal was eliminated by 500-times excess of unmarked oligonucleotides. The dorsal root ganglion
10 sections were hybridized with oligonucleotides A, C or D marked with digoxigenin (DIG)-dUTP and with probe E marked with DIG-dUTP by PCR. The marking of the probes, the preparation of the samples, the hybridization and the visualization of the DIG nucleic acids with alkaline phosphatase conjugated with anti-DIG antibodies were performed in accordance with the
15 supplier's protocols (Boehringer Mannheim).

Computer analysis

The sequence alignments and the phylogenetic tree (Kimura substitution, UPGMA option) were performed with the GCG program (Genetics Computer Group, Madison, WI).

20 *Identification of hASIC3*

Comparison of the rat DRASIC protein sequence with the database of expressed sequence tags (EST) identified two partial cDNA sequences from human total fetus (Genbank accession AA449579 and AA449322).

Both sequences originate from the same clone (IMAGE ID 785700) that we obtained from the UK HGMP RESOURCE CENTRE. Sequencing both strands using an Applied Biosystems automatic sequencer showed that the clone contains the entire coding sequence.

5 *Chromosomal localization*

The human ASIC3 gene was mapped by PCR on the Genebridge 4 Radiation Hybrid DNA panel with the primers CGATTGCAGTTCAGCATCTCT (sense) and ACCATTCGGCAGCCGCACTT (antisense) at an annealing temperature of 65°C. The PCR products were
10 analyzed on 2% agarose gels. Samples were considered positive when a strong amplification of a 159 bp fragment was detected (Code 1), ambiguous when a faint amplification of this fragment was detected (Code 2) and negative when no amplification around 160 bp was visible (Code 0). The positive control (human genomic DNA) was positive and the negative control
15 (hamster genomic DNA) was negative. The following code sequence for the 83 radiation hybrids was obtained and entered into the RHMAPPER program on the Whithead Institute (<http://www-genome.wi.mit.edu>) with a Lod score cutoff of 21: 00000 00100 00001 00021 00100 12010 00000 12112 21000 00001 10120 00010 00102 11010 00010 00212 11011 00001 100.

20 *Expression in COS cells*

The vector containing the hASIC3 coding sequence was linearized with *NotI* and blunt ended with T4 DNA polymerase. After inactivation of the T4 DNA polymerase, the hASIC3 coding sequence was excised with *EcoRI*

and subsequently subcloned into the *EcoRI/SaII* (blunt) digested PCI expression vector (Promega). COS cells, at a density of 20.000 cells per 35 mm diameter petri dish, were transfected with a mix of CD8 and hASIC3-PCI (1:5) using the DEAE-Dextran method. Cells were used for electro-physiological measurements one to three days after transfection. Successfully transfected cells were recognised by their ability to fix CD8-antibody-coated beads [13].

Electrophysiology

Ion currents were recorded using either the whole cell or outside-out patch-clamp technique. The pipette solution contained (in mM): KCl 120, NaCl 30, MgCl₂ 2, EGTA 5, HEPES 10 (pH 7.2). The bath solution contained in mM: NaCl 140, KCl 5, MgCl₂ 2, CaCl₂ 2, HEPES 10 (pH 7.3). Changes in extracellular pH were induced by opening one out of six outlets of a microperfusion system in front of the cell or patch. Test solutions having a pH of less than 6 were buffered with 10 mM MES rather than HEPES but were identical to the control solution in all other respects. Experiments were carried out at room temperature (20-24 °C).

Results

The 35 kb cDNA isolated from rat brain codes for a protein of 526 amino acids that exhibits, as shown in Figure 2, homologies with all of the cloned members of the family of amiloride-sensitive degenerine sodium channels.

As shown in Figure 5, expression of the cRNA in the *Xenopus* oocytes induced an inflowing current activated by H⁺ protons. The biophysical and pharmacological properties of the rASIC1A channel are close to those described for the proton-activated cationic channels of sensory neurons [3, 15, 16]. Reduction of the extracellular pH below a pH of 6.9 activates a rapidly rising and desensitized inflowing current (Figure 5a and b). This channel is activated by extracellular protons since, as shown in Figure 5 (c and d), application of an acid on the extracellular surface of the outside-out patch activates the channel. Intracellular acidification of oocytes and acidification of the intracellular surface of the outside-out patch does not activate the rASIC1A channel nor alter the rASIC1A current induced by the extracellular protons.

The analysis of curves I-V of Figure 5 (c and e) recorded with different extracellular cations shows that Na⁺ is the majority permeable ion (simple conductance channel 14.3 pS). Like the proton-sensitive ionic channel of the sensory neurons [15, 16], the ASIC channel discriminates weakly between the cations (Figure 5c, e, f). In fact, the channel is also permeable to Li⁺, K⁺, Ca²⁺ and H⁺ with the ratios $pNa^+/pLi^+ = 1.3$ (Figure 5c, e), $pNa^+/pK^+ = 13$ (Figure 5c, e), $pNa^+/Ca^{2+} = 2.5$ (Figure 5e) and $pNa^+/H^+ = 0.8$ (Figure 5f). The permeability to Ca²⁺ of ASIC could be a voltage-independent entry path of Ca²⁺ into the cell. An inflowing current of Ca²⁺ into the cell via the ASIC channels can be detected in the absence of extracellular Na⁺ (Figure 6a, b). As indicated in Figure 5 (e), the unitary conductance for Ca²⁺ was 5.2 pS. In

the presence of 140 mM of extracellular Na^+ , augmentation of the concentrations of external Ca^{2+} diminished the amplitude of the current activated by the protons (Figure 6c), thereby demonstrating that Ca^{2+} inhibits the permeability to Na^+ . Blockage by external Ca^{2+} is characteristic of the

5 $I(\text{H}^+)$ of the sensory neurons [17]. The inflowing current activated by H^+ in the sensory neurons is inhibited by amiloride [18] and ethylisopropylamiloride (EIPA) [19]. As shown in Figure 6 (d, e), the rASIC1A channel exhibits the same pharmacology and is blocked in a reversible manner ($K_d = 10 \mu\text{M}$) by amiloride and its derivatives benzamil and EIPA.

10 In addition, the rASIC1A channel protein exhibits approximately 67% sequence homology with the degenerine ionic channel referred to as MDEG [14] or BNaCl [20], herein designated rASIC2. However, the electrophysiological properties of these two clones expressed in *Xenopus* oocytes are clearly different:

15 - As shown in Figure 5(a), the rASIC2 channel is not activated by the same pH changes as the rASIC1A channel.

- Substitution of the glycine residue in position 430 of rASIC2 by an acid-inhibiting amino acid such as valine or phenylalanine activates the channel [14], just as the mutation of alanine in position 704 of degenerine

20 MEC-4 causes neurodegeneration in *C. elegans* [12]. Identical mutations of rASIC1A (glycine in position 431 replaced by valine or phenylalanine) do not lead to activity and the mutants cannot be activated by protons.

Proton-activated cationic channels have been described not only in the sensory neurons but also in the neurons of the central nervous system [21]. The tissue distribution of the expression of the mRNA of the hASIC1A channel is in agreement with this observation. As shown in Figure 7a, a 4.3-
5 kb transcript was detected in the brain by Northern blot analysis and the PT-PCR results presented in Figure 7b show that the dorsal root ganglion expresses the rASIC1A mRNA. Figure 8 (a, b) shows that rASIC1A mRNA is well expressed by the small neurons of the dorsal root ganglion, which supports the fact that ASIC is the rapidly desensitizing proton-activated
10 cationic channel described in the nociceptive sensory neurons. Whereas the presence of proton-activated cationic channels in the dorsal root ganglion is in agreement with their function of acidity detector in nociception, their role in the brain remains to be established. The results of *in-situ* hybridization in Figure 8c show a broad and heterogeneous expression of the rASIC1A
15 channel mRNA. The highest levels of expression were observed in the principal olfactory bulb, the cerebral cortex, the hippocampus, the habenula, the basolateral amygdaloid nucleus and the cerebellum. The synaptic activity accompanies extracellular pH changes [22, 23] and the rapid localized pH changes in or close to the synaptic cleft are noticeably more saturated and
20 stronger than the reported macroscopic fluctuations in the pH.

The proton-activated cationic channels are the only known ionic channels that are directly activated by a change in pH and it was envisaged that the extracellular fluctuations in pH played a neuromodulator role [23].

The expression of cationic channels in the brain supports in addition the hypothesis that the pH fluctuations are not solely a neuronal activation by a product, but even more a communications pathway in the central nervous system.

5 In addition to the rapidly inactivated proton-activated cationic channels, the presence has been reported in the sensory neurons of proton-activated cationic channels exhibiting slower kinetics [4, 24]. The proton-activated cationic channels probably form, like other cationic channels activated by a ligand [25, 26], a family of cationic channels in which different
10 subunits or combinations of subunits constitute channels with diverse pharmacological and biophysical properties.

 The sensation of acidity is not uniquely implicated in nociception but is also associated with the transduction of taste [2]. Acid stimulations activate the proton-activated cationic channels in the taste cells [2, 27] and amiloride
15 inhibits the perception of acid taste [2]. Also, the physiological as well as pharmacological data indicate that rASIC1A and other members of this family are implicated in the transduction of taste. It is, in fact, especially surprising that the same class of ionic channels is associated with different facets of sensory perception:

20 - the amiloride-sensitive sodium channels are associated with the transduction of salty taste [2].

- the degenerines of *C. elegans* are implicated in mechanotransduction and have been proposed as forming the mechanosensitive ionic channels [28, 29].

- the ASIC family of channels are implicated in nociception and the
5 transduction of acid taste.

Comparison of the rASIC3 sequence with the database of expressed sequence tags identified a novel human member of this ion channel family. This novel clone from a total human embryo library codes for a protein of 533 amino acids that shares the closest homology (84% identity, 87% homology)
10 with rASIC3 (Fig. 9). The cloning of a nearly identical cDNA from human testis (hTNaC1), although without functional expression, was reported recently [14].

Expression of the novel hASIC3 clone in COS cells induced a H⁺-gated cation current with kinetics very similar to that of rASIC3. When the pH is
15 decreased rapidly from pH 7.3 to pH 5, a biphasic current is observed. A rapidly inactivating component is followed by a sustained current (Fig. 10A). These very peculiar kinetics that are also found with the rASIC3 [9] channel together with the sequence homology (84% amino acid, 82% nucleic acid identity) with rASIC3 suggest that this novel clone is the human ASIC3. We
20 therefore call it hASIC3 (human Acid Sensing Ion Channel 3).

The pH dependence of the transient hASIC3 current ($pH_{0.5}=6.2$, Fig. 10A) is almost identical to that reported for rASIC3 ($pH_{0.5} = 6.5$) [9]. However, the pH dependencies of the sustained rASIC3 and hASIC3 currents are

clearly different. While rASIC3 requires very acidic pH values ($< \text{pH } 4.5$) [9] for activation of the sustained current, the sustained hASIC3 current starts to activate when the extracellular pH decreases to below pH 6 and reaches half-maximal activity at pH 4.3 (Fig. 10A). The channel activity of hASIC3 depends, just as that of the rASIC3 channel, on the resting pH (Fig. 10B). The maximal activity of the transient hASIC3 current was observed when the resting pH was above pH 8, indicating that a fraction of the transiently activating H^+ -gated cation channels are inactivated at physiological pH. Half-maximal activation of the transient current was observed at pH 7.5, a slightly more alkaline pH than that reported for the rASIC3 clone (pH 6.5) [9]. When the resting pH was below pH 7, only activation of the sustained current could be observed after acidification of the bath medium (Fig. 10B). The sustained hASIC3 current can, just as the sustained rASIC3 channel, still be activated when the initial pH is quite acidic (pH5) (Fig. 10B).

All members of the ASIC family cloned so far are sensitive to the diuretic amiloride. The hASIC3 channel is no exception. The effect of amiloride on the hASIC3 current is similar to that reported for rASIC3 [9]. The transient current is inhibited by amiloride ($K_D = 15.9 \mu\text{M}$; Fig. 2C) as well as by triamterene (Fig. 10C), while the sustained hASIC3 current is virtually not affected by those diuretics.

The transient hASIC3 current reverses at 37.6 mV, close to the Na^+ reversal potential, indicating a high selectivity for Na^+ vs K^+ (Fig. 11A). Conversely, the sustained current discriminates much less between Na^+ and

K⁺ (selectivity ratio $g_{Na^+}/g_{K^+} = 1.62$) as it reverses at 10.1 mV (Fig. 11A). The low selectivity for Na⁺ vs K⁺ of the sustained hASIC3 current clearly distinguishes the hASIC3 channel from the rASIC3 channel which is highly selective for Na⁺ [9].

5 Proton-induced unitary currents were recorded from excised outside-out patches (Fig. 11B-D). In a narrow pH window around pH 7.3, spontaneous channel activity can be observed (Fig. 11C) that disappears upon an increase in pH to 8.0, a decrease in pH to 6.0 (not shown) or in the presence of 100 μ M amiloride (Fig. 11C). This basal current is mainly carried
10 by Na⁺, since it reverses at 30.2 mV (Fig. 11B). When the pH on the extracellular face of an outside-out patch is decreased from pH 7.3 to pH 4, unitary currents are induced (Fig. 11D) that reverse at the same membrane potential as the spontaneously active channel (Fig. 11B). The unitary conductance of the hASIC3 channel for Na⁺ is 15 ± 0.6 pS, close to that
15 reported for rat ASIC3 (12.6 pS) [9]. While the sustained non-selective H⁺-activated hASIC3 current could be easily detected in whole cell recordings, no sustained or non-selective current could be recorded on outside-out patches. One possible explanation is, that soluble factors might be necessary that are lost during excision of the patch.

20 The human chromosomal localization of the hASIC3 gene was determined by PCR on a human-hamster radiation hybrid DNA panel. The hASIC3 gene is localised on the human chromosome 7q35, 6.4 cRad telomeric from the microsatellite AFMA082XC9 (Lod score > 21). To our

knowledge, no hereditary diseases with symptoms that are consistent with an altered function of a H⁺-gated cation channel were mapped to this region of the human genome.

- The hASIC3 channel subunit forms a sustained H⁺-gated cation
- 5 channel that has properties similar to those reported for the rASIC3 channel. However, very important differences exist. Most importantly, the sustained hASIC3 current requires less acidic pH for activation than rASIC3 [9]. In this respect the properties of the hASIC3 channel match better the physiological and electrophysiological data from sensory neurones than those of rASIC3.
- 10 Subcutaneous perfusion of human volunteers with acidic buffer causes pain. At pH 5.2, the pain was rated 20% on a scale ranging from 0 to 100% (unbearable pain) [2]. Furthermore, a subpopulation of polymodal C-fibres in rat nerve-skin preparations can be excited by acidic pH [4]. The threshold for activation lies between pH 6.9 and pH 6.1, maximal stimulation is reached at
- 15 pH 5.2. The endogenous H⁺-gated cation channel recorded in rat sensory neurones starts to activate below pH 6.6 [5]. The pH dependence of the sustained hASIC3 current matches closely those physiological data, while rASIC3 has a pH dependence that is shifted two pH units towards more acidic pH values [9]. One possible explanation for the differences between
- 20 physiological data and the pH dependence of the sustained ASIC3 channel (especially the rASIC3) might be the participation of as yet unknown subunits in the formation of the native channel. Heteromultimeric assembly was previously demonstrated for the rASIC3 channel [9]. rASIC3 can associate

with rASIC2b resulting in an altered selectivity of the channel. While rASIC3 is completely Na⁺-selective, the sustained current of the heteromultimeric rASIC3/rASIC2b channel does not discriminate between Na⁺ and K⁺. The H⁺-gated cation channel recorded in rat sensory neurones does not discriminate

5 between Na⁺ and K⁺ either [5], suggesting that both rASIC3 and rASIC2b participate in the formation of this ion channel in rat sensory neurons. In contrast with the rASIC3 channel, hASIC3 does not require coexpression of other subunits to generate a non-selective sustained current. The ion selectivity of sustained human H⁺-gated cation channels is not known yet. A

10 more detailed electrophysiological characterization of human sustained H⁺-gated cation channels will be necessary to allow a comparison of the properties of the native channel with those of the hASIC3 channel.

List of Sequences

INFORMATION CONCERNING SEQ ID NO: 1

- 15 i) CHARACTERISTIC OF THE SEQUENCE:
- A) LENGTH: 3562 base pairs
- B) TYPE: nucleic acid
- C) NUMBER OF STRANDS: double
- D) CONFIGURATION: linear
- 20 ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: rat
- ix) CHARACTERISTIC
- A) NAME/KEY: ASIC

B) LOCALIZATION: 123 .. 1700

xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 1:

Top of page 28 =

5 **INFORMATION CONCERNING SEQ ID NO: 2**

i) CHARACTERISTIC OF THE SEQUENCE:

A) LENGTH: 1620 base pairs

B) TYPE: nucleic acid

C) NUMBER OF STRANDS: double

10 D) CONFIGURATION: linear

ii) TYPE OF MOLECULE: DNA

vi) ORIGIN: human

ix) CHARACTERISTIC

A) NAME/KEY: ASIC

15 B) LOCALIZATION: 1 .. 1542

xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 2:

Top of page 31 =

INFORMATION CONCERNING SEQ ID NO: 3

20 i) CHARACTERISTIC OF THE SEQUENCE:

A) LENGTH: 1666 base pairs

B) TYPE: nucleic acid

C) NUMBER OF STRANDS: double

- D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: human
- ix) CHARACTERISTIC
- 5 A) NAME/KEY: MDEG
- B) LOCALIZATION: 127 .. 1663
- xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 3:

Top of page 34 =

- 10 INFORMATION CONCERNING SEQ ID NO: 4
- i) CHARACTERISTIC OF THE SEQUENCE:
 - A) LENGTH: 3647 base pairs
 - B) TYPE: nucleic acid
 - C) NUMBER OF STRANDS: double
 - 15 D) CONFIGURATION: linear
 - ii) TYPE OF MOLECULE: DNA
 - vi) ORIGIN: rat
 - ix) CHARACTERISTIC
 - A) NAME/KEY: ASIC1B
 - 20 B) LOCALIZATION: 109 .. 1785
 - xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 4:

Top of page 38 =

INFORMATION CONCERNING SEQ ID NO: 5

- i) CHARACTERISTIC OF THE SEQUENCE:
 - A) LENGTH: 1602 base pairs
 - 5 B) TYPE: nucleic acid
 - C) NUMBER OF STRANDS: double
 - D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: rat
- 10 ix) CHARACTERISTIC
 - A) NAME/KEY: ASIC3
 - B) LOCALIZATION: 1 .. 1602
- xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 5:

Top of page 41 =

15 **INFORMATION CONCERNING SEQ ID NO: 6**

- i) CHARACTERISTIC OF THE SEQUENCE:
 - A) LENGTH: 1948 base pairs
 - B) TYPE: nucleic acid
 - C) NUMBER OF STRANDS: double
 - 20 D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: rat
- ix) CHARACTERISTIC

- A) NAME/KEY: ASIC2B
- B) LOCALIZATION: 16 .. 1707
- xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 6:

5

INFORMATION CONCERNING SEQ ID NO: 7

- i) CHARACTERISTIC OF THE SEQUENCE:
 - A) LENGTH: 1736 base pairs
 - B) TYPE: nucleic acid
 - C) NUMBER OF STRANDS: double
 - 10 D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: human
- ix) CHARACTERISTIC
 - A) NAME/KEY: ASIC3
 - 15 B) LOCALIZATION: 18 .. 1611
 - xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 7:

INFORMATION CONCERNING SEQ ID NO: 8

- i) CHARACTERISTIC OF THE SEQUENCE:
 - A) LENGTH: 531
 - 20 B) TYPE: protein
 - C) NUMBER OF STRANDS: single
 - D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: protein

- vi) ORIGIN: human
- ix) CHARACTERISTIC
- A) NAME/KEY: hASIC3
- B) LOCALIZATION: 1 - 531

5

REFERENCES

1. Rang, H.P., Bevan, S. & Dray, A. *Br. Med. Bull.* 47, 534-548 (1991).
- 10 2. Lindeman, B. *Physiol. Rev.* 76, 718-766 (1996).
3. Krishtal, O.A. & Pidoplichko, V.I. *Neuroscience* 6, 2599-2601 (1981).
- 15 4. Bevan, S. & Geppeti, P. *Trends Neurosci.* 17, 509-512 (1994).
5. Akaike, N., Krishtal, O.A. & Maruyama, T. *J. Neurophysiol.* 63, 805-813 (1990).
- 20 6. Canessa, C.M., Horisberger, J.D. & Rossier, B.C. *Nature* 361, 467-470 (1993).
7. Canessa, C.M., Schild, L., Buell, G., Thorens, B., Gautschi, I., Horisberger, J.D. & Rossier, B.C. *Nature* 367, 463-467 (1994).
- 25 8. Lingueglia, E., Voilley, N., Waldmann, H., Lazunski, M. & Barbry, P. *Febs Lett.* 318, 95-99 (1993).
9. Lingueglia, E., Renard, S., Waldmann, R., Voilley, N., Champigny, G., Plass, H., Lazunski, M. & Barbry, P., *J. Biol. Chem.* 269, 13736-13739 (1994)..
- 30 10. Lingueglia, E., Champigny, G., Lazdunski, M. & Barbry, P. *Nature* 378, 730-733 (1995).
- 35 11. Waldmann, R., Champigny, G., Bassilana, F., Voilley, N. & Lazdunski, M. *J. Biol. Chem.* 270, 27411-27414 (1995).
12. Driscoll, M. & Chalfie, M. *Nature* 349, 588-593 (1991).

13. Huang, M. & Chalfie, M. *Nature* 367, 467-470 (1994).
- 5 14. Waldmann, R., Champigny, G., Voilley, N., Lauritzen, I. & Lazdunski, M. *J. Biol. Chem.* 271, 10433-10434 (1996).
- 15 15. Kovalchuk Yu, N., Krishtal, O.A. & Nowycky, M.C. *Neurosci. Lett.* 115-237-242 (1990).
- 10 16. Konnerth, A., Lux, H.D. & Morad, M. *J. Physiol.* 386, 603-633 (1987).
17. Davies, N.W., Lux, H.D. & Morad, M. *J. Physiol.* 400, 159-187 (1988).
- 15 18. Korkushko, A.O. & Krishtal, O.A. *Neirofiziologiya* 16, 557-561 (1984).
19. Grantyn, R., Perouansky, M., Rodriguez-Tebar, A. & Lux, H.D. *Dev. Brain Res.* 49, 150-155 (1989).
- 20 20. Price, M.P., Snyder, P.M. & Welsh, M.J. *J. Biol. Chem.* 271, 7879-7882 (1996).
- 25 21. Akaike, N. & Ueno, S. *Prog. Neurobiol.* 43, 73-83 (1994).
22. Krishtal, O.A., Osipchuk, Y.V., Shelest, T.N. & Smirnoff, S.V. *Brain Res.* 436, 352-356 (1987).
- 30 23. Chesler, M. & Kaila, K. *Trends Neurosci.* 15, 396-402 (1992).
24. Bevan, S. & Yeats, J. *J. Physiol.* 433, 145-161 (1991).
- 35 25. Lewis, C., Neidhart, S., Holy, C., North, R.A., Buell, G. & Surprenant, A. *Nature* 377, 432-435 (1995).
26. Barnard, E.A. *Trends Pharmacol. Sci.* 17, 305-309 (1996).
- 40 27. Okada, Y., Miyamoto, T. & Sato, T. *J. Exp. Biol.* 187, 19-32 (1994).
28. Liu, J. Schrank, B. & Waterson, R. *Science* 273, 361 (1996).
- 45 29. Waldmann, R., Champigny, G. & Lazdunski, M. *J. Biol. Chem.* 270, 11735-11737 (1995).

30. Renard, S., Lingueglia, E., Voilley, N., Lazdunski, M. & Barbry, P. *J. Biol. Chem.* 269, 12981-12986 (1994).
- 5 31. Reeh, P.W. and Steen, K.H. *Prog Brain Res* 113, 143-151 (1996).
32. Steen, K.H., Steen, A.E., Kreysel, H.W. & Reeh, P.W. *Pain* 66, 163-170 (1996).
- 10 33. Steen, K.H., Issberner, U. & Reeh, P.W. *Neurosci Lett* 199, 29-32 (1995).
34. Steen, K.H., Reeh, P.W., Anton, F. & Handwerker, H.O. *J. Neurosci* 12, 86-95 (1992).
- 15 35. Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C. and Lazdunski, M. *Nature* 386, 173-177 (1997).
- 20 36. Bassilana, F., Champigny, G., Waldmann, R., de Weille, J.R., Heurteaux, C. & Lazdunski, M. *J. Biol. Chem.* 272, 28819-28822 (1997).
- 25 37. Waldmann, R., Bassilana, F., de Weille, J., Champigny, G., Heurteaux, C. & Lazdunski, M. *J. Biol. Chem.* 272, 20975-20978 (1997).
- 30 38. Lingueglia, E., de Weille, J.R., Bassilana, F., Heurteaux, C., Sakai, H., Waldmann, R. & Lazdunski, M. *J. Biol Chem.* 272, 29778-29783 (1997).
39. Waldmann, R. & Lazdunski, M. *Curr. Op. Neurobiol.* 8, 418-424 (1998).
- 35 40. Baumann, T.K., Burchiel, K.J., Ingram, S.L. & Martenson, M.E. *Pain* 65, 31-38 (1996).
41. Jurman, M.E., Boland, L.M., Liu, Y. & Yellen, G. *Biotechniques* 17, 876-881 (1994).
- 40 42. Ishibashi, K. & Marumo, F. *Biochem. Biophys Res Comm* 245, 589-593 (1998).

We claim:

1. An isolated and purified nucleic acid molecule encoding the human cation transport protein ASIC3.
2. The sequence of claim 1 which maps to chromosome 7q35.
- 5 3. The sequence of claim 1 represented by SEQ ID No. 7.
4. An isolated and purified human cation transport protein represented by SEQ ID No. 8.
5. The protein of claim 4 which comprises a proton gated cation channel.
- 10 6. The protein of claim 5 wherein the transport channel activity is dependent upon resting pH.
7. The protein of claim 5 which also exhibits biphasic desensitization kinetics.
8. The protein of claim 4 which is sensitive to amiloride.
- 15 9. A human cation transport channel comprising at least one molecule of ASIC3 and at least one molecule of a second human proton-gated cation channel.
10. The cation transport channel of claim 9 wherein the second cation transport protein is selected from the group consisting of hASIC2A, hASIC2B,
20 hASIC1A and hASIC1B.
11. A cloning vehicle comprising the sequence of claim 1.

12. The cloning vehicle of claim 11 which also comprises a sequence encoding a second cation transport protein selected from the group consisting of hASIC2A, hASIC2B, hASIC1A and hASIC1B.
13. A transformed cell containing the cloning vehicle of claim 11.
- 5 14. The transformed cell of claim 13 which expresses hASIC3.
15. A transformed cell containing the cloning vehicle of claim 12.
16. The transformed cell of claim 15 which expresses a human cation transport channel comprising at least one molecule of ASIC3 and at least one molecule of a second cation transport protein selected from the group
- 10 consisting of hASIC2A, hASIC2B, hASIC1A and hASIC1B.
17. A method of screening for substances capable of modulating the activity of cation transport channels comprised of hASIC3, comprising contacting pre-selected amounts of the substance to be tested with cells expressing said cation transport channel, measuring the effects of the
- 15 substance on the transport functions of the cation transport channel, and identifying the substance that has a positive or negative effect on potassium channel activity .
18. A substance, identified by the method of claim 20 that is capable of positively or negatively influencing the transport functions of a cation
- 20 transport channel.

1/17

Met Glu Leu Lys Thr Glu Glu Glu Glu Val Gly Gly Val Gln Pro Val Ser Ile
Pro Val Ser Ile

Gln Ala Phe Ala Ser Ser Ser Thr Leu His Gly Leu Ala His Ile Phe Ser Tyr
Gln Ala Phe Ala Ser Ser Ser Thr Leu His Gly Met Ala His Ile Phe Ser Tyr

Glu Arg Leu Ser Leu Lys Arg Ala Leu Trp Ala Leu Cys Phe Leu Gly Ser Leu
Glu Arg Leu Ser Leu Lys Arg Ala Leu Trp Ala Leu Cys Phe Leu Gly Ser Leu

Ala Val Leu Leu Cys Val Cys Thr Glu Arg Val Gln Tyr Tyr Phe Cys Tyr His
Ala Val Leu Leu Cys Val Cys Thr Glu Arg Val Gln Tyr Tyr Phe His Tyr His

His Val Thr Lys Leu Asp Glu Val Ala Ala Ser Gln Leu Thr Phe Pro Ala Val
His Val Thr Lys Leu Asp Glu Val Ala Ala Ser Gln Leu Thr Phe Pro Ala Val

Thr Leu Cys Asn Leu Asn Glu Phe Arg Phe Ser Gln Val Ser Lys Asn Asp Leu
Thr Leu Cys Asn Leu Asn Glu Phe Arg Phe Ser Gln Val Ser Lys Asn Asp Leu

Tyr His Ala Gly Glu Leu Leu Ala Leu Leu Asn Asn Arg Tyr Glu Ile Pro Asp
Tyr His Ala Gly Glu Leu Leu Ala Leu Leu Asn Asn Arg Tyr Glu Ile Pro Asp

Thr Gln Met Ala Asp Glu Lys Gln Leu Glu Ile Leu Gln Asp Lys Ala Asn Phe
Thr Gln Met Ala Asp Glu Lys Gln Leu Glu Ile Leu Gln Asp Lys Ala Asn Phe

Arg Ser Phe Lys Pro Lys Pro Phe Asn Met Arg Glu Phe Tyr Asp Arg Ala Gly
Arg Ser Phe Lys Pro Lys Pro Phe Asn Met Arg Glu Phe Tyr Asp Arg Ala Gly

His Asp Ile Arg Asp Met Leu Leu Ser Cys His Phe Arg Gly Glu Ala Cys Ser
His Asp Ile Arg Asp Met Leu Leu Ser Cys His Phe Arg Gly Glu Val Cys Ser

Ala Glu Asp Phe Lys Val Val Phe Thr Arg Tyr Gly Lys Cys Tyr Thr Phe Asn
Ala Glu Asp Phe Lys Val Val Phe Thr Arg Tyr Gly Lys Cys Tyr Thr Phe Asn

Ser Gly Gln Asp Gly Arg Pro Arg Leu Lys Thr Met Lys Gly Gly Thr Gly Asn
Ser Gly Arg Asn Gly Arg Pro Arg Leu Lys Thr Met Lys Gly Gly Thr Gly Asn

Gly Leu Glu Ile Met Leu Asp Ile Gln Gln Asp Glu Tyr Leu Pro Val Trp Gly
Gly Leu Glu Ile Met Leu Asp Ile Gln Gln Asp Glu Tyr Leu Pro Val Trp Gly

Glu Thr Asp Glu Thr Ser Phe Glu Ala Gly Ile Lys Val Gln Ile His Ser Gln
Glu Thr Asp Glu Thr Ser Phe Glu Ala Gly Ile Lys Val Gln Ile His Ser Gln

Asp Glu Pro Pro Phe Ile Asp Gln Leu Gly Phe Gly Val Ala Pro Gly Phe Gln
Asp Glu Pro Pro Phe Ile Asp Gln Leu Gly Phe Gly Val Ala Pro Gly Phe Gln

Figure 1

2/17

Thr Phe Val Ser Cys Gln Glu Gln Arg Leu Ile Tyr Leu Pro Ser Pro Trp Gly
Thr Phe Val Ala Cys Gln Glu Gln Arg Leu Ile Tyr Leu Pro Pro Pro Trp Gly
 Thr Cys Asn Ala Val Thr Met Asp Ser Asp Phe Phe Asp Ser Tyr Ser
Thr Cys Lys Ala Val Thr Met Asp Ser Asp Leu Asp Phe Phe Asp Ser Tyr Ser
 Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Leu Val Glu Asn Cys Asn
Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Leu Val Glu Asn Cys Asn
 Cys Arg Met Val His Met Pro Gly Asp Ala Pro Tyr Cys Thr Pro Glu Gln Tyr
Cys Arg Met Val His Met Pro Gly Asp Ala Pro Tyr Cys Thr Pro Glu Gln Tyr
 Lys Glu Cys Ala Asp Pro Ala Leu Asp Phe Leu Val Glu Lys Asp Gln Glu Tyr
Lys Glu Cys Ala Asp Pro Ala Leu Asp Phe Leu Val Glu Lys Asp Gln Glu Tyr
 Cys Val Cys Glu Met Pro Cys Asn Leu Thr Arg Tyr Gly Lys Glu Leu Ser Met
Cys Val Cys Glu Met Pro Cys Asn Leu Thr Arg Tyr Gly Lys Glu Leu Ser Met
 Val Lys Ile Pro Ser Lys Ala Ser Ala Lys Tyr Leu Ala Lys Lys Phe Asn Lys
Val Lys Ile Pro Ser Lys Ala Ser Ala Lys Tyr Leu Ala Lys Lys Phe Asn Lys
 Ser Glu Gln Tyr Ile Gly Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Val
Ser Glu Gln Tyr Ile Gly Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Val
 Leu Asn Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Ile Ala Gly Leu Leu
Leu Asn Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Ile Ala Gly Leu Leu
 Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu Thr Val
Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu Thr Val
 Leu Glu Leu Phe Asp Tyr Ala Tyr Glu Val Ile Lys His Arg Leu Cys Arg Arg
Leu Glu Leu Phe Asp Tyr Ala Tyr Gly Val Ile Lys His Lys Leu Cys Arg Arg
 Gly Lys Cys Gln Lys Glu Ala Lys Arg Ser Ser Ala Asp Lys Gly Val Ala Leu
Gly Lys Cys Gln Lys Glu Ala Lys Arg Ser Ser Ala Asp Lys Gly Val Ala Leu
 Ser Leu Asp Asp Val Lys Arg His Asn Pro Cys Glu Ser Leu Arg Gly His Pro
Ser Leu Asp Asp Val Lys Arg His Asn Pro Cys Glu Ser Leu Arg Gly His Pro
 Ala Gly Met Thr Tyr Ala Ala Asn Ile Leu Pro His His Pro Ala Arg Gly Thr
Ala Gly Met Thr Tyr Ala Ala Asn Ile Val Pro His His Pro Ala Arg Gly Thr
Phe Glu Asp Phe Thr Cys
 Phe Glu Asp Phe Thr Cys

Fig 1 (continued)

3/17

[illegible]

Figure 2

ASIC	311	DCETRYIVENCNCRMVHMPGDAP	CTPEQ
MDEG	310	DCETRYIVENCNCRMVHMPGDAP	CTPEQ
FaNaCh	325	LCKQRLIIRRCGCGSSAIPFVPS	NATFCGVIKDWQEI NRNHSNEDHNQSEEDRAFIPTP
MEC-4	586	SCFQQLIKKRCRCGDPFIFPVEG	..ARHCDAADPVARR
ASIC	340	YKECAIPALDFLVEKD..QEYCNCEMPCNLTRYGKELSMVKIP	..
MDEG	339	HKECAEPALGLLAEKD..SNYCCRTPCNLTRYNKELSMVKIP	..
FaNaCh	385	YLACEEEREQKLNNDRTYELSCGCFQPCSETSYLKSISYWP	LEFYQLSAVERFFKQER
MEC-4	622	CLARMNDLGGLHG...SFRRCRCQQPCGQIYSVTYSPAKWPSLSLQIQLG	..
ASIC	381	..SKASAKYLAKKFNKSEQ	YI GENI
MDEG	380	..SKTSAKYLEKKFNKSEK	..YI SENI
FaNaCh	445	QAGQNHFMKTYYEYLEKLAHPSTKHLARNDSHMDDILSKSYSLSEKEMAKEASDILRN	..
MEC-4	670SCNGTAVECNKHYKENG
M II			
ASIC	404	LVLDIFFEVLNYETIEQKKAYEIA..LLGDI GGQMG LFI GAS	LTLELFDYAYEIKHLL
MDEG	403	LVLDIFFEVLNYETIEQKKAYEIA..LLGDI GGQMG LFI GAS	LTLELFDYAYEIKHLL
FaNaCh	505	LRLIILEEDLSVVEYRQLPAYGADLF..DIDIGGTGLWGGIS	LTILELTLIRLTGLVF
MEC-4	687	AMVAVFVEQLNEMETSEAYGFVNLLDFGGQLGLWCGIS	FLTCCEFFVFLFL ETAYMSA
ASIC	484	CRGKCQKEAKRSSADKGMALSLDDVKRHNPCFSGIRGHPAGMYANLPHHPARGTFF	FFC
MDEG	463	..LDLKGKEEEEGSHDENMS...TCTCPHSETSHVN..PLQTALGTLE	FFC
FaNaCh	585	NSEKGLPFGPTTVNNNGSNHNSQSTSQHQLYGYMCHDSDSHYS	DAGASVDFDRRGVESP
MEC-4	747	EHNYSLYK KKKAEKAKKVASGSF	..

Fig.2 (continued)

5/17

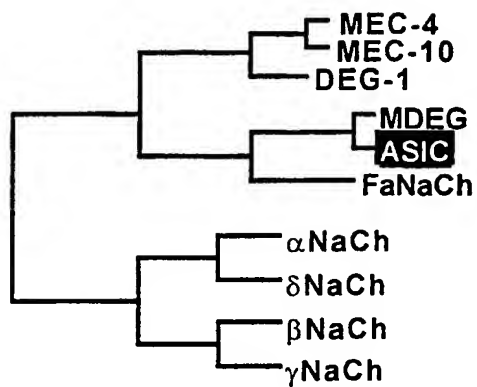


Figure 3

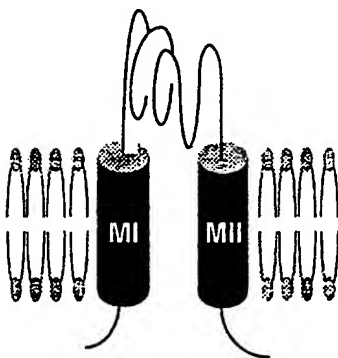


Figure 4

6/17

Fig. 5 a

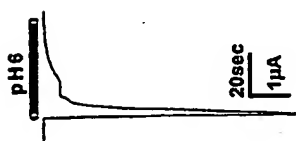


Fig. 5 b

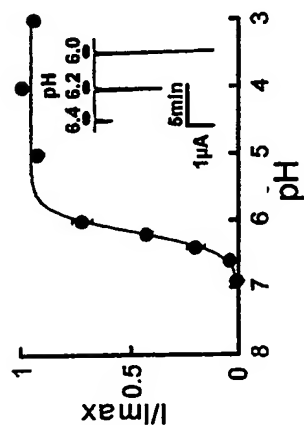


Fig. 5 c

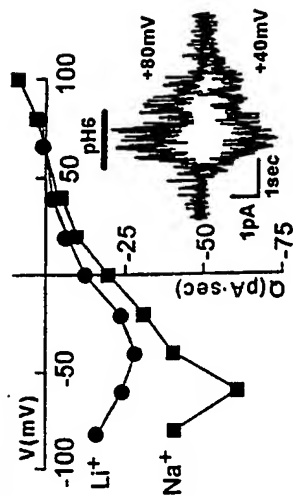


Fig. 5 d

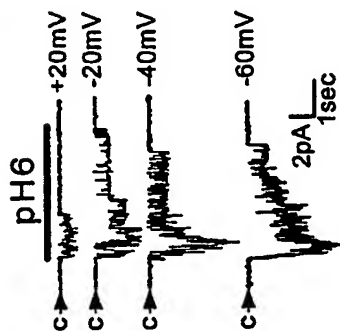


Fig. 5 e

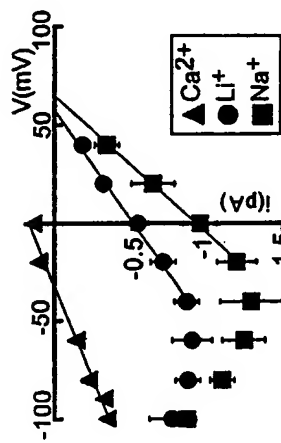
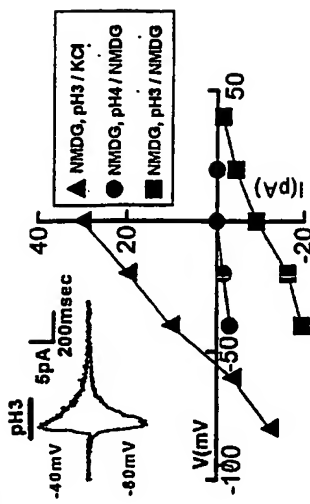
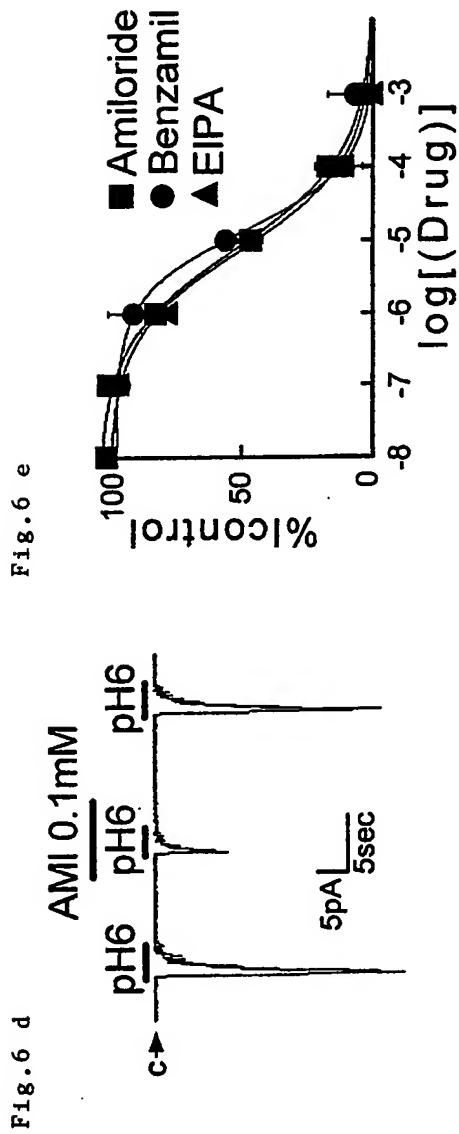
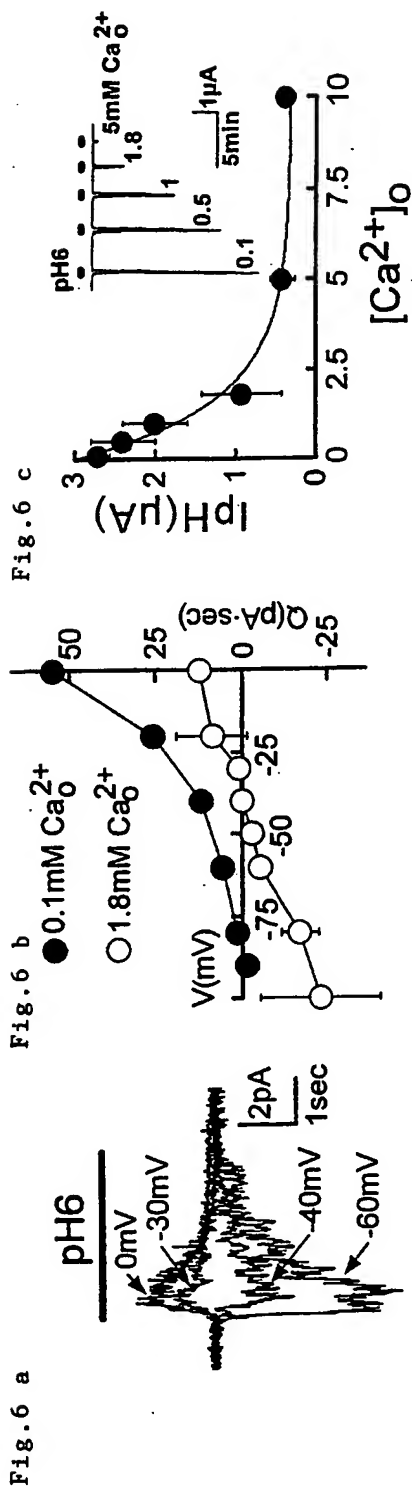


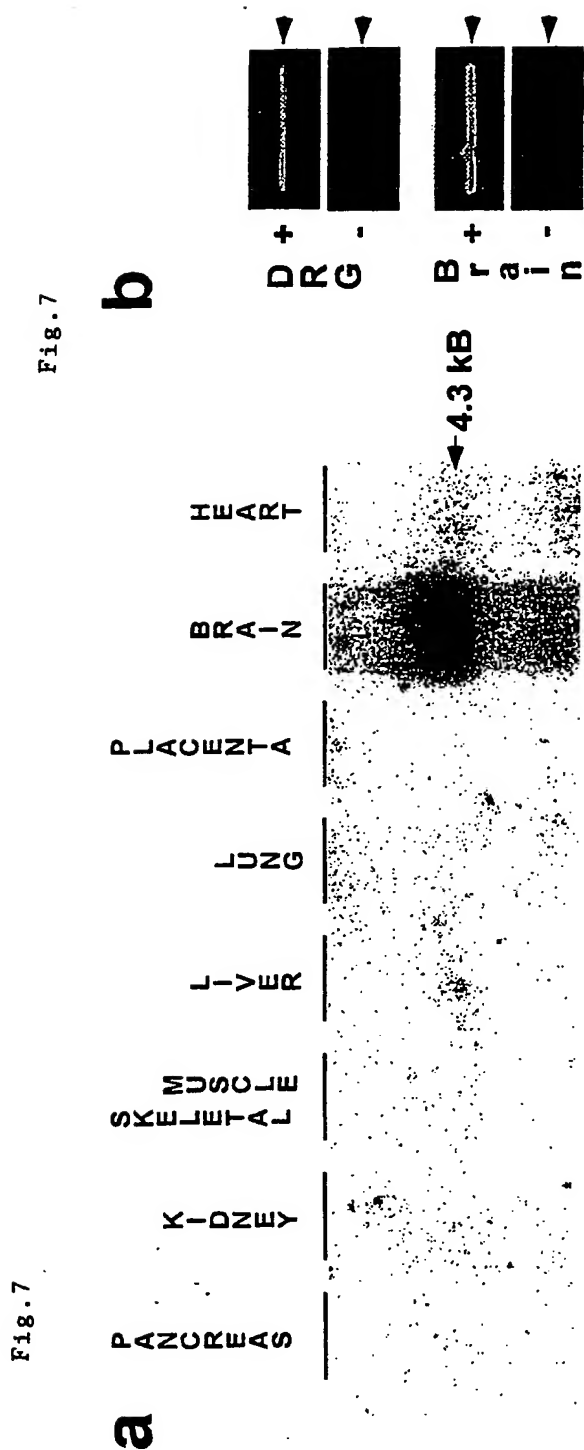
Fig. 5 f



7/17



8/17



9/17

Fig.8 a

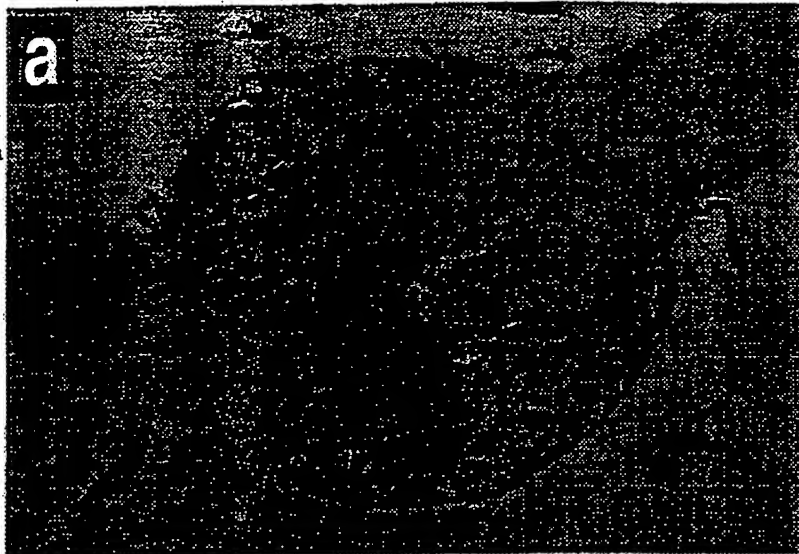


Fig.8 b

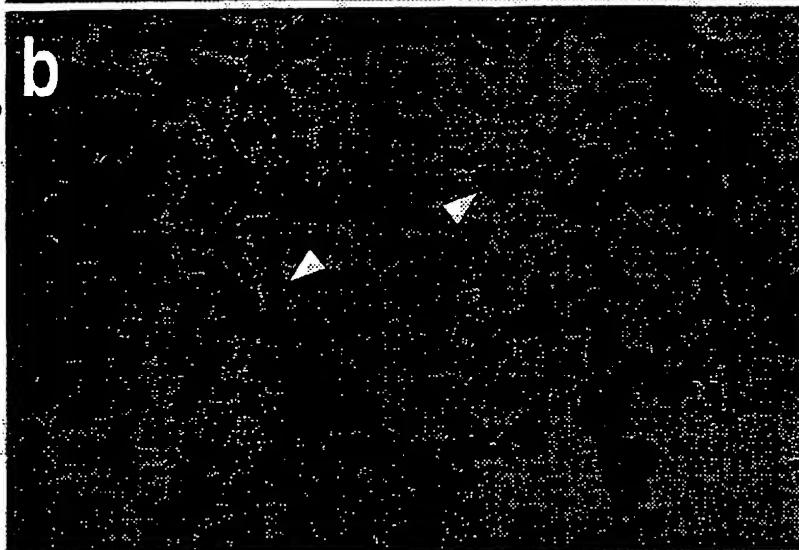
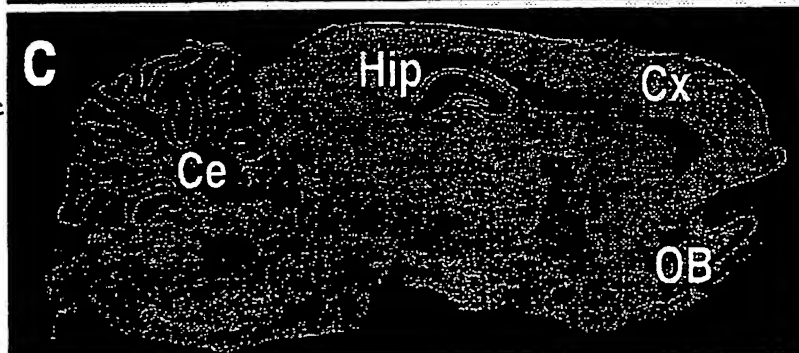


Fig.8 c



10/17

basic3	1	MKP	TSG	PEEA	-RR	PAS	DIR	VFAS	NC	MMH	G	L	G	H	V	E	G	P	G	S	L	S	L	R	R	G	M	A	A	A	V	L	S	V	A	T	E	L	Y	Q																					
basic3	1	MKP	R	S	G	L	E	E	A	O	R	R	O	A	S	D	I	R	V	F	A	S	S	C	M	H	G	L	G	H	I	F	G	P	G	L	T	L	R	R	G																				
basic3	60	V	A	E	R	V	R	Y	Y	R	E	F	H	H	Q	T	A	L	D	E	R	E	S	H	R	L	I	F	P	A	V	T	L	C	N	I	N	P	L	R	R	S	R	L	T	P	N	D	L	H	W	A	G	S	A	L	L	G	L	D	P
basic3	61	V	A	E	R	V	R	Y	Y	G	E	E	H	H	K	T	T	L	D	E	R	E	S	H	Q	L	T	E	P	A	V	T	L	C	N	I	N	P	L	R	R	S	R	L	T	P	N	D	L	H	W	A	G	T	A	L	L	G	L	D	P
basic3	120	A	E	H	A	A	F	L	R	A	L	G	R	P	P	A	P	P	G	F	M	P	S	P	T	F	D	M	A	Q	L	Y	A	R	A	G	H	S	L	D	M	L	D	C	R	F	R	G	Q	P	C	G	P	E	N	F	T	I			
basic3	121	A	E	H	A	A	F	L	R	A	L	G	R	P	P	A	P	P	G	F	M	P	S	P	T	F	D	M	A	Q	L	Y	A	R	A	G	H	S	L	D	M	L	D	C	R	F	R	G	Q	P	C	G	P	E	N	F	T	I			
basic3	180	F	T	R	M	G	K	C	Y	T	F	N	S	G	A	D	G	A	E	L	L	T	T	P	K	G	G	A	G	N	G	L	D	I	M	L	D	V	Q	Q	E	E	Y	L	P	V	M	R	D	N	E	E	T	P	F	E	V	G	I	R	
basic3	181	F	T	R	M	G	O	C	Y	T	F	N	S	G	A	H	G	A	E	L	L	T	T	P	K	G	G	A	G	N	G	L	D	I	M	L	D	V	Q	Q	E	E	Y	L	P	V	M	R	D	N	E	E	T	P	F	E	V	G	I		
basic3	240	Q	I	H	S	O	E	E	P	P	I	I	D	O	L	G	P	G	A	P	G	H	Q	T	F	V	S	C	Q	Q	Q	L	S	F	L	P	P	P	W	G	D	C	S	S	A	S	L	N	P	N	-Y	E	P	E	P	S	D				
basic3	241	Q	I	H	S	O	E	E	P	P	I	I	D	O	L	G	P	G	A	P	G	H	Q	T	F	V	S	C	Q	Q	Q	L	S	F	L	P	P	P	W	G	D	C	S	S	A	S	L	N	P	N	-Y	E	P	E	P	S	D				
basic3	299	L	G	S	P	S	P	P	Y	T	L	M	G	C	R	L	A	C	E	T	R	Y	V	A	R	K	C	G	C	R	M	V	M	P	G	D	V	P	V	C	S	P	Q	Q	Y	K	N	C	A	H	P	A	I	D	A						
basic3	301	L	G	S	P	R	P	S	P	P	Y	S	L	I	G	C	R	L	A	C	E	T	R	Y	V	A	R	K	C	G	C	R	M	H	M	P	G	N	S	P	V	C	S	P	Q	Q	Y	K	N	C	A	H	P	A	I	D					
basic3	359	L	R	K	D	S	C	A	C	P	N	P	C	A	S	T	R	Y	A	K	E	L	S	M	V	R	I	P	S	R	A	A	R	F	L	A	R	K	L	N	R	S	E	A	Y	I	A	E	N	V	L	A	L	D	I	F	F	E			
basic3	361	L	R	K	D	T	C	V	C	P	N	P	C	A	T	T	R	Y	A	K	E	L	S	M	V	R	I	P	S	R	A	A	R	F	L	A	R	K	L	N	R	S	E	A	Y	I	A	E	N	V	L	A	L	D	I	F	F				
basic3	419	N	Y	E	T	V	E	Q	K	A	Y	E	M	S	E	L	L	G	D	I	G	G	Q	M	G	L	F	I	G	A	S	L	L	T	I	L	E	I	L	D	Y	L	C	E	V	F	R	D	K	V	L	G	Y	F	W	N	R	Q			
basic3	421	N	Y	E	T	V	E	Q	K	A	Y	E	M	S	E	L	L	G	D	I	G	G	Q	M	G	L	F	I	G	A	S	L	L	T	I	L	E	I	L	D	Y	L	C	E	V	F	R	D	K	V	L	G	Y	F	W						
basic3	479	Q	P	H	S	T	N	L	L	Q	E	G	L	C	S	H	R	T	Q	V	P	H	L	S	L	G	P	R	P	P	T	P	P	C	A	V	T	K	T	L	S	A	S	H	R	T	C	Y	L	V	T										
basic3	481	Q	P	H	S	T	N	L	L	Q	E	G	L	C	S	H	R	T	Q	V	P	H	L	S	L	G	P	R	P	P	T	P	P	C	A	V	T	K	T	L	S	A	S	H	R	T	C	Y	L	V											

Figure 9

11/17

Fig.9 continued

Human ASIC3 sequence

10 30 50
ACGACGGGGTTCTGGCCATGAAGCCCACCTCAGGCCAGAGGAGGCCCGGCCAGCCT
M K P T S G P E E A R R P A S

70 90 110
CGGACATCCGCGTGTTCGCCAGCAACTGCTCGATGCACGGGCTGGGCCACGTCTTCGGGC
D I R V F A S N C S M H G L G H V F G P

130 150 170
CAGGCAGCCTGAGCCTGCGCCGGGGGATGTGGGCAGCGGCCGTGGTCCTGTCAGTGGCCA
G S L S L R R G M W A A A V V L S V A T

190 210 230
CCTTCCTCTACCAGGTGGCTGAGAGGGTGCGCTACTACAGGGAGTTCCACCACCAGACTG
F L Y Q V A E R V R Y Y R E F H H Q T A

250 270 290
CCCTGGATGAGCGAGAAAGCCACCGGCTCATCTTCCCGGCTGTCACCCTGTGCAACATCA
L D E R E S H R L I F P A V T L C N I N

310 330 350
ACCCACTGCGCCGCTCGCGCCTAACGCCCAACGACCTGCACTGGGCTGGGTCTGCGCTGC
P L R R S R L T P N D L H W A G S A L L

370 390 410
TGGGCCTGGATCCCGCAGAGCACGCCGCCTTCCTGCGCGCCCTGGGCCGGCCCCCTGCAC
G L D P A E H A A F L R A L G R P P A P

12/17

Fig.9 continued

430 450 470
CGCCCCGGCTTCATGCCCAGTCCCACCTTTGACATGGCGCAACTCTATGCCCCGTGCTGGGC
P G F M P S P T F D M A Q L Y A R A G H

490 510 530
ACTCCCTGGATGACATGCTGCTGGACTGTCGCTTCCGTGGCCAACCTTGTGGGCCTGAGA
S L D D M L L D C R F R G Q P C G P E N

550 570 590
ACTTCACCACGATCTTCACCCGGATGGGAAAGTGCTACACATTTAACTCTGGCGCTGATG
F T T I F T R M G K C Y T F N S G A D G

610 630 650
GGGCAGAGCTGCTCACCCTACTAGGGGTGGCATGGGCAATGGGCTGGACATCATGCTGG
A E L L T T T R G G M G N G L D I M L D

670 690 710
ACGTGCAGCAGGAGGAATATCTACCTGTGTGGAGGGACAATGAGGAGACCCCGTTTGAGG
V Q Q E E Y L P V W R D N E E T P F E V

730 750 770
TGGGGATCCGAGTGCAGATCCACAGCCAGGAGAGCCGCCCATCATCGATCAGCTGGGCT
G I R V Q I H S Q E E P P I I D Q L G L

790 810 830
TGGGGGTGTCCCCGGGCTACCAGACCTTTGTTTCTTGCCAGCAGCAGCAGCTGAGCTTCC
G V S P G Y Q T F V S C Q Q Q Q L S F L

850 870 890
TGCCACCGCCCTGGGGCGATTGCAGTTCAGCATCTCTGAACCCCAACTATGAGCCAGAGC

13/17

Fig.9 continued

P P P W G D C S S A S L N P N Y E P E P

910 930 950

CCTCTGATCCCCTAGGCTCCCCCAGCCCCAGCCCCAGCCCTCCCTATACCCTTATGGGGT

S D P L G S P S P S P P Y T L M G C

970 990 1010

GTCGCCTGGCCTGCGAAACCCGCTACGTGGCTCGGAAGTGC GGCTGCCGAATGGTGTACA

R L A C E T R Y V A R K C G C R M V Y M

1030 1050 1070

TGCCAGGCGACGTGCCAGTGTGCAGCCCCCAGCAGTACAAGAACTGTGCCACCCGGCCA

P G D V P V C S P Q Q Y K N C A H P A I

1090 1110 1130

TAGATGCCATGCTTCGCAAGGACTCGTGC GCCTGCCCCAACCCGTGCGCCAGCACGCGCT

D A M L R K D S C A C P N P C A S T R Y

1150 1170 1190

ACGCCAAGGAGCTCTCCATGGTGC GGATCCCGAGCCGCGCCGCGCGCTTCCTGGCCC

A K E L S M V R I P S R A A A R F L A R

1210 1230 1250

GGAAGCTCAACCGCAGCGAGGCCTACATCGCGGAGAACGTGCTGGCCCTGGACATCTTCT

K L N R S E A Y I A E N V L A L D I F F

1270 1290 1310

TTGAGGCCCTCAACTATGAGACCGTGGAGCAGAAGAAGGCCTATGAGATGTCAGAGCTGC

E A L N Y E T V E Q K K A Y E M S E L L

14/17

Fig.9 continued

1330 1350 1370
TTGGTGACATTGGGGGCCAGATGGGGCTGTTTCATCGGGGCCAGCCTGCTCACCATCCTCG
G D I G G Q M G L F I G A S L L T I L E

1390 1410 1430
AGATCCTAGACTACCTCTGTGAGGTGTTCCGAGACAAGGTCCTGGGATATTTCTGGAACC
I L D Y L C E V F R D K V L G Y F W N R

15/17

Fig.10 a

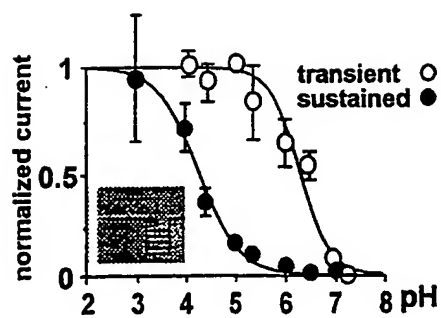
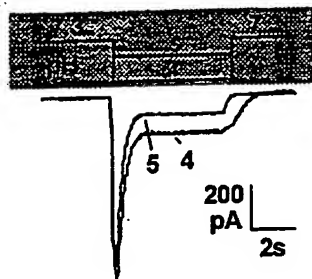


Fig.10 b

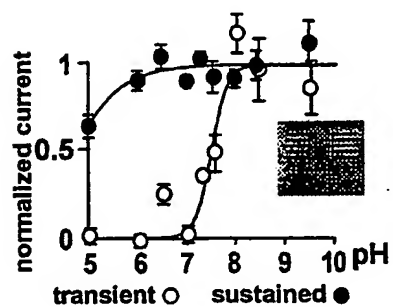
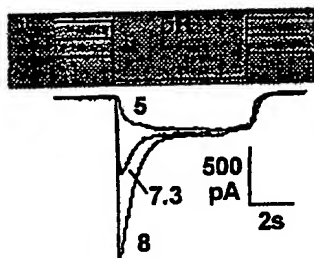
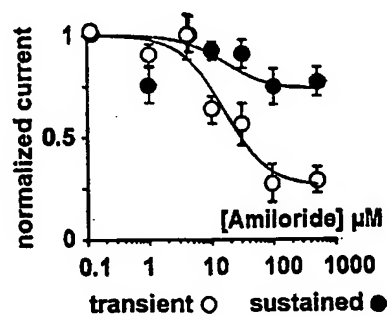
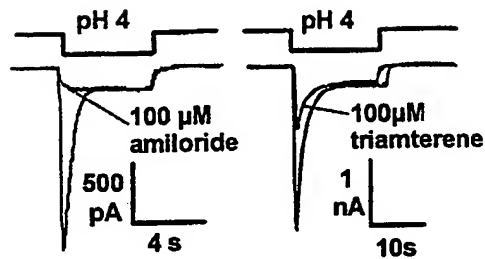


Fig.10 c



16/17

Fig.11 a

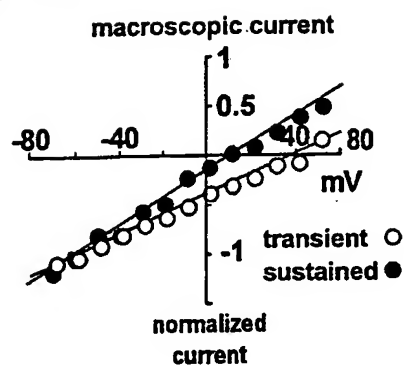


Fig.11 b

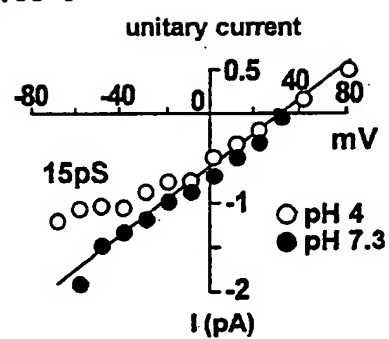


Fig.11 c

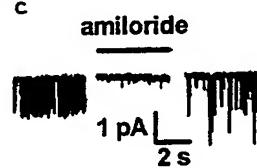
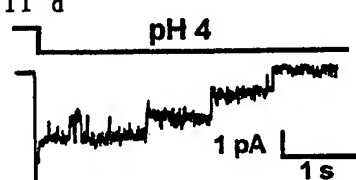


Fig.11 d



17/17

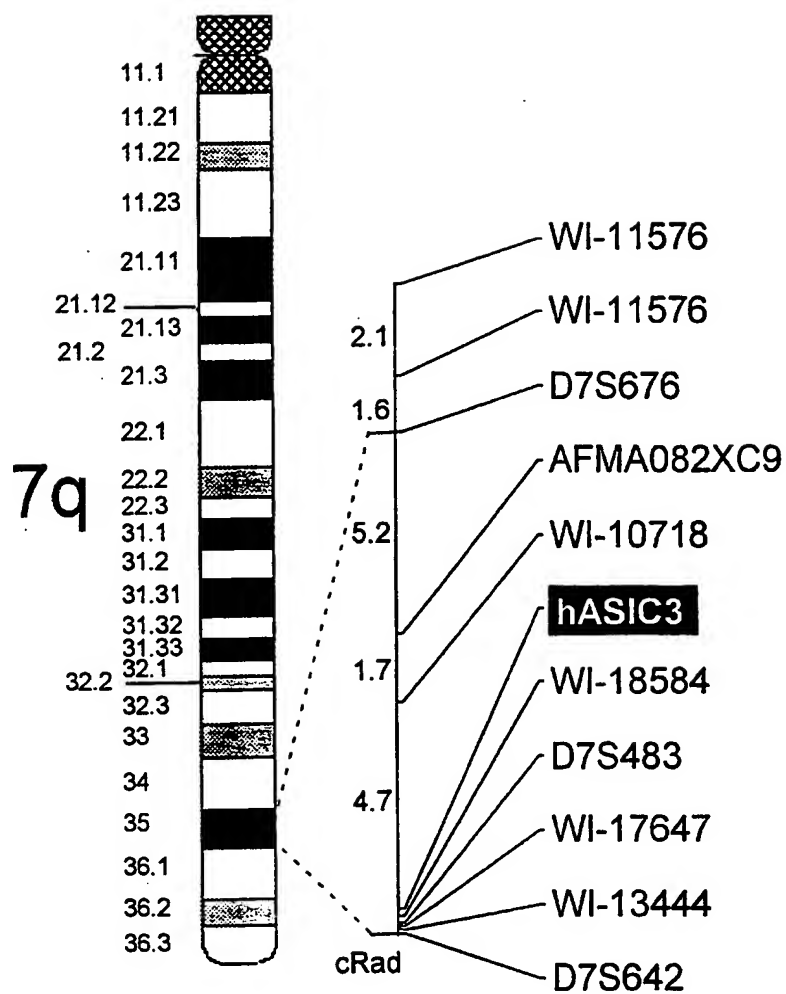


Figure 12

LISTE DE SÉQUENCES.

NOMBRE DE SÉQUENCES : 8

5 INFORMATION CONCERNANT LA SEQ ID NO:1 :

- i) CARACTÉRISTIQUE DE LA SÉQUENCE :
 A) LONGUEUR : 3562 paires de base
 B) TYPE : acide nucléique
 10 C) NOMBRE DE BRINS : double
 D) CONFIGURATION : linéaire

ii) TYPE DE MOLECULE : ADN

vi) ORIGINE : rat

15

ix) CARACTÉRISTIQUE

A) NOM/CLE : ASIC

B) LOCALISATION : 123 .. 1700

20 xi) DESCRIPTION DE LA SÉQUENCE : SEQ ID NO:1 :

	CACACACACA CACACACACA CACACACACA CACACACACA CACACAGAAC CTGCGCCTGT	60
	GCCTGTGCCT GTGCCTGTGC CTGTTTGAGA GCTGGAGACA CAGAAGGATC CCCTTGCCAA	120
25	GG ATG GAA TTG AAG ACC GAG GAG GAG GAG GTG GGT GGT GTC CAG CCG Met Glu Leu Lys Thr Glu Glu Glu Glu Val Gly Gly Val Gln Pro 1 5 10 15	167
30	GTG AGC ATC CAG GCT TTC GCC AGC AGC TCC ACG CTG CAT GGT CTT GCC Val Ser Ile Gln Ala Phe Ala Ser Ser Thr Leu His Gly Leu Ala 20 25 30	215
35	CAC ATC TTC TCC TAT GAG CGG CTG TCT CTG AAG CGG GCA CTG TGG GCC His Ile Phe Ser Tyr Glu Arg Leu Ser Leu Lys Arg Ala Leu Trp Ala 35 40 45	263
40	CTG TGC TTC CTG GGT TCG CTG GCC GTC CTG CTG TGT GTG TGC ACT GAG Leu Cys Phe Leu Gly Ser Leu Ala Val Leu Leu Cys Val Cys Thr Glu 50 55 60	311
45	CGT GTG CAG TAC TAC TTC TGC TAT CAC CAC GTC ACC AAG CTT GAC GAA Arg Val Gln Tyr Tyr Phe Cys Tyr His His Val Thr Lys Leu Asp Glu 65 70 75	359
50	GTG GCT GCC TCC CAG CTC ACC TTC CCT GCT GTC ACA CTG TGC AAT CTC Val Ala Ala Ser Gln Leu Thr Phe Pro Ala Val Thr Leu Cys Asn Leu 80 85 90 95	407
55	AAT GAG TTC CGC TTT AGC CAA GTC TCC AAG AAT GAC CTG TAC CAT GCT Asn Glu Phe Arg Phe Ser Gln Val Ser Lys Asn Asp Leu Tyr His Ala 100 105 110	455
	GGG GAG CTG CTG GCC CTG CTC AAC AAC AGG TAT GAG ATC CCG GAC ACA Gly Glu Leu Leu Ala Leu Leu Asn Asn Arg Tyr Glu Ile Pro Asp Thr 115 120 125	503

	CAG ATG GCT GAT GAA AAG CAG CTA GAG ATA TTG CAG GAC AAG GCC AAC	551
	Gln Met Ala Asp Glu Lys Gln Leu Glu Ile Leu Gln Asp Lys Ala Asn	
	130 135 140	
5	TTC CGG AGC TTC AAG CCC AAG CCC TTC AAC ATG CGT GAA TTC TAC GAC	599
	Phe Arg Ser Phe Lys Pro Lys Pro Phe Asn Met Arg Glu Phe Tyr Asp	
	145 150 155	
10	AGA GCG GGG CAC GAT ATT CGA GAC ATG CTG CTC TCG TGC CAC TTC CGT	647
	Arg Ala Gly His Asp Ile Arg Asp Met Leu Ser Cys His Phe Arg	
	160 165 170 175	
15	GGG GAG GCC TGC AGC GCT GAA GAT TTC AAA GTG GTC TTC ACT CGG TAT	695
	Gly Glu Ala Cys Ser Ala Glu Asp Phe Lys Val Val Phe Thr Arg Tyr	
	180 185 190	
20	GGG AAG TGT TAC ACA TTC AAC TCG GGC CAA GAT GGG CGG CCA CGG CTG	743
	Gly Lys Cys Tyr Thr Phe Asn Ser Gly Gln Asp Gly Arg Pro Arg Leu	
	195 200 205	
25	AAG ACC ATG AAA GGT GGG ACT GGC AAT GGC CTG GAG ATC ATG CTG GAC	791
	Lys Thr Met Lys Gly Gly Thr Gly Asn Gly Leu Glu Ile Met Leu Asp	
	210 215 220	
30	ATT CAG CAA GAT GAA TAT TTG CCT GTG TGG GGA GAG ACC GAC GAG ACA	839
	Ile Gln Gln Asp Glu Tyr Leu Pro Val Trp Gly Glu Thr Asp Glu Thr	
	225 230 235	
35	TCC TTC GAA GCA GGC ATC AAA GTG CAG ATC CAC AGT CAG GAT GAA CCC	887
	Ser Phe Glu Ala Gly Ile Lys Val Gln Ile His Ser Gln Asp Glu Pro	
	240 245 250 255	
40	CCT TTC ATC GAC CAG CTG GGC TTT GGT GTG GCT CCA GGT TTC CAG ACG	935
	Pro Phe Ile Asp Gln Leu Gly Phe Gly Val Ala Pro Gly Phe Gln Thr	
	260 265 270	
45	TTT GTG TCT TGC CAG GAG CAG AGG CTC ATC TAC CTG CCC TCA CCC TGG	983
	Phe Val Ser Cys Gln Glu Gln Arg Leu Ile Tyr Leu Pro Ser Pro Trp	
	275 280 285	
50	GGC ACC TGC AAT GCT GTT ACC ATG GAC TCG GAT TTC TTC GAC TCC TAC	1031
	Gly Thr Cys Asn Ala Val Thr Met Asp Ser Asp Phe Phe Asp Ser Tyr	
	290 295 300	
55	AGC ATC ACT GCC TGC CGG ATT GAT TGC GAG ACG CGT TAC CTG GTG GAG	1079
	Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Leu Val Glu	
	305 310 315	
60	AAC TGC AAC TGC CGT ATG GTG CAC ATG CCA GGG GAC GCC CCA TAC TGC	1127
	Asn Cys Asn Cys Arg Met Val His Met Pro Gly Asp Ala Pro Tyr Cys	
	320 325 330 335	
65	ACT CCA GAG CAG TAC AAG GAG TGT GCA GAT CCT GCC CTG GAC TTC CTA	1175
	Thr Pro Glu Gln Tyr Lys Glu Cys Ala Asp Pro Ala Leu Asp Phe Leu	
	340 345 350	
70	GTG GAG AAA GAC CAG GAA TAC TGC GTG TGT GAG ATG CCT TGC AAC CTG	1223
	Val Glu Lys Asp Gln Glu Tyr Cys Val Cys Glu Met Pro Cys Asn Leu	
	355 360 365	

	ACC CGC TAC GGC AAG GAG CTG TCC ATG GTC AAG ATC CCA AGC AAA GCC	1271
	Thr Arg Tyr Gly Lys Glu Leu Ser Met Val Lys Ile Pro Ser Lys Ala	
	370 375 380	
5	TCC GCC AAG TAC CTG GCC AAG AAG TTC AAC AAA TCG GAG CAG TAC ATA	1319
	Ser Ala Lys Tyr Leu Ala Lys Lys Phe Asn Lys Ser Glu Gln Tyr Ile	
	385 390 395	
10	GGG GAG AAC ATT CTG GTG CTG GAC ATT TTC TTT GAA GTC CTC AAC TAT	1367
	Gly Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Val Leu Asn Tyr	
	400 405 410 415	
15	GAG ACC ATC GAG CAG AAA AAG GCC TAT GAG ATC GCA GGG CTG TTG GGT	1415
	Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Ile Ala Gly Leu Leu Gly	
	420 425 430	
20	GAC ATC GGG GGC CAG ATG GGG TTG TTC ATC GGT GCC AGC ATC CTC ACC	1463
	Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu Thr	
	435 440 445	
25	GTG CTG GAA CTC TTT GAC TAT GCC TAC GAG GTC ATT AAG CAC AGG CTG	1511
	Val Leu Glu Leu Phe Asp Tyr Ala Tyr Glu Val Ile Lys His Arg Leu	
	450 455 460	
30	TGC AGA CGT GGA AAG TGC CAG AAG GAG GCT AAG AGG AGC AGC GCA GAC	1559
	Cys Arg Arg Gly Lys Cys Gln Lys Glu Ala Lys Arg Ser Ser Ala Asp	
	465 470 475	
35	AAG GGC GTG GCG CTC AGC CTG GAT GAC GTC AAA AGA CAC AAT CCC TGC	1607
	Lys Gly Val Ala Leu Ser Leu Asp Asp Val Lys Arg His Asn Pro Cys	
	480 485 490 495	
40	GAG AGC CTC CGA GGA CAT CCT GCC GGG ATG ACG TAC GCT GCC AAC ATC	1655
	Glu Ser Leu Arg Gly His Pro Ala Gly Met Thr Tyr Ala Ala Asn Ile	
	500 505 510	
45	CTA CCT CAC CAT CCC GCT CGA GGC ACG TTT GAG GAC TTT ACC TGC TAA	1703
	Leu Pro His His Pro Ala Arg Gly Thr Phe Glu Asp Phe Thr Cys *	
	515 520 526	
50	GCCCTCGCAG GCCGCTGTAC CAAAGGCCCTA GGTGGGGAGG GCTGGGGGAG CAAGGGGCCC	1763
	CCAAGTCCCC CCAGCTACCC TGTGGACTTA ACTGCATTCC TGGTCAGTGG TTCCCTCTTG	1823
55	TCTGTGGTGA GAAAGGAGTC TTGACCATAG AGTCCTCTCC CAGCCTCTAT CCCATCTTTT	1943
	TATTTTAATT TAATCACATT TGCTCTGTAA TATTGCTTGA GGCTGGGGAT CGTGATTTC	2003
	CCCCAGTTCT TTTATTGTTG AGAATAGTTT TCTCTATTCT GGGTTTCTG TTATTTCAAA	2063
	TGAATCTGCA AATTGCTCTT CCCATCTCTA TGAAGAATTG CGTTGGAATT TTGATGGGGA	2123
	TTGTATTGAA TCTGTAGATT GCCTTTGGTA AGATGGCCAT TTTTACTATG TTAATCCTGC	2183
	CAATTCATGA GCAAGGGAGA TCTTTCTATC TCTGAAATCT ACTTCAGTTT CTTTCTTCAG	2243
	AGACTTGAAG TTCTTGTCAT AAAAATCTTT TTGGTTAGAG CCACACCAAG GTATTTTATA	2303
	TTGTTTGTGA CTATTGTGAA TGGTGTCAAT TCCCTAATTT CCTTCTCAGC CTACTTATCC	2363

	TTTGAGTAGA GGAAGGCTTC TGATTTGTTT GGGTTAATTT TATACCCAGC TGCTTTGCTA	2423
	AAGTTCTTTA TCAGGTTTAG GTGTCTCTCG GTGGAACTTT TGGGGTCACG TAAGAATACT	2483
5	ATTATATCAT CTGCAAATAG TGATATTTCA CTTCTTCCTT TCCAATTTCT ATCCCTCTGG	2543
	GGACTTTTGG TTGTCTAATT GCTCTGGCTA GGACTTCAAA TTCTATATTG AATAGATAGG	2603
10	GAGAGAGTGG GCAGCCTTGT CTAGTTCTCG GTTTTCGTGG GATCGCTTCA AATTTCTCTC	2663
	CATTTAGTTT GATATTGGCT ACTGGTTTGC TGTATATGGC TTTTACTGTA CTTAGGTATG	2723
	GGCCTTGAAT TCCTGATATT TCCAAGACTT TTAACATGAA GGGGTTTTGA AATTTGCCAA	2783
15	ATGCTTTCTC AGCATCTAAT GAGATGATCA TGTGCCCTCC CCCCACCTTG AGTTTGTTTA	2843
	TATAGTGGGT TACATGAAAG GATCATTCTT AATAGTCCAC AAGTCTGCCA AATCTTGCTG	2903
20	ATTGTGACTC ATTTCCATAG CAGGCTCTAT AACTTCTCTA ACAGATTGCA TTAAACTCTG	2963
	CTTGGGGAAG GCATTACCTC TTGGTTGAAG CAATGTTGTA GTTCTATGC CTGCTGAGTA	3023
	AATAGCCTCA AGTCCAAGTA CTTGCCCAGA CTAATGATCA AACGTATCCA GGAGTTCCAT	3083
25	ACCAGAGATG TACTCTTCTC TCCTTTGAAG TACATTGCTG GAAGAGTAAT TGTGTTTGCT	3143
	AGAGATACTC CTTGAACTG CAAAAGAAAT CTCTTGGCTA AGCATATAAT CAAGCCTCAG	3203
30	GTTTTCTTTT TATTAAATAG CTGCTTGTA GAAAGTGGAC ACTAAGCATA TACCTCAAAG	3263
	GGAGACAGAA TGACTCTGTG CCTTCACTGA TGGAAGTCTG GGTTACAAAT TACATCAGAA	3323
	GAACCTATCA TAGTGAAACA TCTCATTCCC CTGGTATAAT CCCTTCTAGA AATACACTTG	3383
35	TGACTCTGAA ATGTTATAAT CGTGACAAC TGGCTGTTAC AGATACACCA AGTTAAATTT	3443
	GATAGAGAAA CCAGGCTTGG AGCCTCATGT CCATAGGGCA AGAGGAAGAT GCTGAGTGT	3503
40	TAAGGTTGGT TTGAGCGAAG AACAATACCT TGTGTCACAA AAATGAAAGG AAAAAAGAAA	3563
	AAAGGAAAGA AGGAAAGAAA GAGAGAGAAA GAAAAAGAAA GAAAGAAAAA AAAAAAAA	3562

INFORMATION CONCERNANT LA SEQ ID NO:2 :

i) CARACTERISTIQUE DE LA SEQUENCE :

5 A) LONGUEUR : 1620 paires de base

B) TYPE : acide nucléique

C) NOMBRE DE BRINS : double

D) CONFIGURATION : linéaire

ii) TYPE DE MOLECULE : ADN

10 vi) ORIGINE : homme

ix) CARACTERISTIQUE

A) NOM/CLE : ASIC

B) LOCALISATION : 1 .. 1542

15 xi) DESCRIPTION DE LA SEQUENCE : SEQ ID NO:2 :

20	CCG GTG AGC ATC CAG GCC TTC GCC AGC AGC TCC ACA CTG CAC GGC ATG Pro Val Ser Ile Gln Ala Phe Ala Ser Ser Ser Thr Leu His Gly Met 1 5 10 15	48
25	GCC CAC ATC TTC TCC TAC GAG CGG CTG TCT CTG AAG CGG GCA CTG TGG Ala His Ile Phe Ser Tyr Glu Arg Leu Ser Leu Lys Arg Ala Leu Trp 20 25 30	96
30	GCC CTG TGC TTC CTG GGC TCG CTG GCT GTG CTG CTG TGT GTG TGC ACG Ala Leu Cys Phe Leu Gly Ser Leu Ala Val Leu Leu Cys Val Cys Thr 35 40 45	144
35	GAG CGT GTG CAG TAC TAC TTC CAC TAC CAC CAT GTC ACC AAG CTC GAC Glu Arg Val Gln Tyr Tyr Phe His Tyr His His Val Thr Lys Leu Asp 50 55 60	192
40	GAG GTG GCT GCC TCT CAG CTT ACC TTC CCT GCT GTC ACG CTG TGC AAC Glu Val Ala Ala Ser Gln Leu Thr Phe Pro Ala Val Thr Leu Cys Asn 65 70 75 80	240
45	CTC AAC GAG TTC CGC TTT AGC CAA GTC TCC AAG AAT GAC CTG TAT CAT Leu Asn Glu Phe Arg Phe Ser Gln Val Ser Lys Asn Asp Leu Tyr His 85 90 95	288
50	GCT GGG GAG CTG CTG GCC CTG CTC AAC AAC AGG TAT GAG ATA CCA GAC Ala Gly Glu Leu Leu Ala Leu Leu Asn Asn Arg Tyr Glu Ile Pro Asp 100 105 110	336
55	ACA CAG ATG GCA GAT GAA AAG CAG CTG GAG ATA CTG CAG GAC AAA GCC Thr Gln Met Ala Asp Glu Lys Gln Leu Glu Ile Leu Gln Asp Lys Ala 115 120 125	384
60	AAC TTC CGC AGC TTC AAA CCC AAA CCC TTC AAC ATG CGT GAG TTC TAC Asn Phe Arg Ser Phe Lys Pro Lys Pro Phe Asn Met Arg Glu Phe Tyr 130 135 140	432
65	GAC CGA GCT GGG CAC GAC ATT CGA GAC ATG CTG CTC TCC TGC CAC TTC Asp Arg Ala Gly His Asp Ile Arg Asp Met Leu Leu Ser Cys His Phe 145 150 155 160	480

	CGG GGG GAG GTC TGC AGC GCT GAA GAC TTC AAG GTG GTC TTC ACA CGC	528
	Arg Gly Glu Val Cys Ser Ala Glu Asp Phe Lys Val Val Phe Thr Arg	
	165 170 175	
5	TAT GGA AAG TGC TAC ACG TTC AAC TCG GGC CGA AAT GGG CGG CCG CGG	576
	Tyr Gly Lys Cys Tyr Thr Phe Asn Ser Gly Arg Asn Gly Arg Pro Arg	
	180 185 190	
10	CTG AAG ACC ATG AAG GGT GGG ACG GGC AAT GGG CTG GAA ATC ATG CTG	624
	Leu Lys Thr Met Lys Gly Gly Thr Gly Asn Gly Leu Glu Ile Met Leu	
	195 200 205	
15	GAC ATC CAG CAG GAC GAG TAC CTG CCT GTG TGG GGG GAG ACT GAC GAG	672
	Asp Ile Gln Gln Asp Glu Tyr Leu Pro Val Trp Gly Glu Thr Asp Glu	
	210 215 220	
20	ACG TCT TTC GAA GCA GGC ATC AAA GTG CAG ATC CAT AGT CAG GAT GAA	720
	Thr Ser Phe Glu Ala Gly Ile Lys Val Gln Ile His Ser Gln Asp Glu	
	225 230 235 240	
25	CCT CCT TTC ATC GAC CAG CTG GGC TTT GGC GTG GCC CCA GGC TTC CAG	768
	Pro Pro Phe Ile Asp Gln Leu Gly Phe Gly Val Ala Pro Gly Phe Gln	
	245 250 255	
30	ACC TTT GTG GCC TGC CAG GAG CAG CGG CTC ATA TAC CTG CCC CCA CCC	816
	Thr Phe Val Ala Cys Gln Glu Gln Arg Leu Ile Tyr Leu Pro Pro Pro	
	260 265 270	
35	TGG GGC ACC TGC AAA GCT GTT ACC ATG GAC TCG GAT TTG GAT TTC TTC	864
	Trp Gly Thr Cys Lys Ala Val Thr Met Asp Ser Asp Leu Asp Phe Phe	
	275 280 285	
40	GAC TCC TAC AGC ATC ACT GCC TGC CGC ATC GAC TGT GAG ACG CGC TAC	912
	Asp Ser Tyr Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr	
	290 295 300	
45	CTG GTG GAG AAC TGC AAC TGC CGC ATG GTG CAC ATG CCA GGG GAT GCC	960
	Leu Val Glu Asn Cys Asn Cys Arg Met Val His Met Pro Gly Asp Ala	
	305 310 315 320	
50	CCA TAC TGT ACT CCA GAG CAG TAC AAG GAG TGT GCA GAT CCT GCT CTG	1008
	Pro Tyr Cys Thr Pro Glu Gln Tyr Lys Glu Cys Ala Asp Pro Ala Leu	
	325 330 335	
55	GAC TTC CTG GTG GAG AAG GAC CAG GAG TAC TGC GTG TGT GAA ATG CCT	1056
	Asp Phe Leu Val Glu Lys Asp Gln Glu Tyr Cys Val Cys Glu Met Pro	
	340 345 350	
60	TGC AAC CTG ACC CGC TAT GGC AAA GAG CTG TCC ATG GTC AAG ATC CCC	1104
	Cys Asn Leu Thr Arg Tyr Gly Lys Glu Leu Ser Met Val Lys Ile Pro	
	355 360 365	
65	AGC AAA GCC TCA GCC AAG TAC CTG GCC AAG AAG TTC AAC AAA TCT GAG	1152
	Ser Lys Ala Ser Ala Lys Tyr Leu Ala Lys Lys Phe Asn Lys Ser Glu	
	370 375 380	
70	CAA TAC ATA GGG GAG AAC ATC CTG GTG CTG GAC ATT TTC TTT GAA GTC	1200
	Gln Tyr Ile Gly Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Val	
	385 390 395 400	

WO 00/08149

PCT/IB99/01445

	CTC AAC TAT GAG ACC ATT GAA CAG AAG AAG GCC TAT GAG ATT GCA GGG	1248
	Leu Asn Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Ile Ala Gly	
	405 410 415	
5	CTC CTG GGT GAC ATC GGG GGC CAG ATG GGG CTG TTC ATC GGG GCC AGC	1296
	Leu Leu Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser	
	420 425 430	
10	ATC CTC ACG GTG CTG GAG CTC TTT GAC TAC GCC TAC GGG GTC ATT AAG	1344
	Ile Leu Thr Val Leu Glu Leu Phe Asp Tyr Ala Tyr Gly Val Ile Lys	
	435 440 445	
15	CAC AAG CTG TGC CGA CGA GGA AAA TGC CAG AAG GAG GCC AAA AGG AGC	1392
	His Lys Leu Cys Arg Arg Gly Lys Cys Gln Lys Glu Ala Lys Arg Ser	
	450 455 460	
20	AGT GCG GAC AAG GGC GTG GCC CTC AGC CTG GAC GAC GTC AAA AGA CAC	1440
	Ser Ala Asp Lys Gly Val Ala Leu Ser Leu Asp Asp Val Lys Arg His	
	465 470 475 480	
	AAC CCG TGC GAG AGC CTT CGG GGC CAC CCT GCC GGG ATG ACA TAC GCT	1488
	Asn Pro Cys Glu Ser Leu Arg Gly His Pro Ala Gly Met Thr Tyr Ala	
	485 490 495	
25	GCC AAC ATC GTA CCT CAC CAT CCG GCC CGA GGC ACG TTC GAG GAC TTT	1536
	Ala Asn Ile Val Pro His His Pro Ala Arg Gly Thr Phe Glu Asp Phe	
	500 505 510	
30	ACC TGC TGA GCGCCGACAGG CCGCCGAACC AAAGACCTAG ATGGGGAGGA CTAGGAGAGC	1595
	Thr Cys *	
	514	
35	GAGGGGGCCC CCAGCTGCCT CCTAA	1620

INFORMATION CONCERNANT LA SEQ ID NO:3 :

- i) CARACTERISTIQUE DE LA SEQUENCE :
- A) LONGUEUR : 1666 paires de base
- 5 B) TYPE : acide nucléique
- C) NOMBRE DE BRINS : double
- D) CONFIGURATION : linéaire
- ii) TYPE DE MOLECULE : ADN
- 10 vi) ORIGINE : homme
- ix) CARACTERISTIQUE
- A) NOM/CLE : MDEG
- B) LOCALISATION : 127 .. 1663
- 15 xi) DESCRIPTION DE LA SEQUENCE : SEQ ID NO:3 :
- | | | |
|----|--|-----|
| | TCTGGCGCGA TGCTTACCTT GCGTTCTCTC CCCTGAACGT CAAGGTTTAA GCAGAGCCCCG | 60 |
| 20 | AGGACTGGGA GCTCTTCTCT GAAATTCGAT CAACCTGAAG CCAGTTGCGG AACTGCACGG | 120 |
| | GGTCCCG ATG GAC CTC AAG GAA AGC CCC AGT GAG GGC AGC CTG CAA CCT | 169 |
| | Met Asp Leu Lys Glu Ser Pro Ser Glu Gly Ser Leu Gln Pro | |
| | 1 5 10 | |
| 25 | TCT AGC ATC CAG ATC TTT GCC AAC ACC TCC ACC CTC CAT GGC ATC CGC | 217 |
| | Ser Ser Ile Gln Ile Phe Ala Asn Thr Ser Thr Leu His Gly Ile Arg | |
| | 15 20 25 30 | |
| 30 | CAC ATC TTC GTG TAT GGG CCG CTG ACC ATC CGG CGT GTG CTG TGG GCA | 265 |
| | His Ile Phe Val Tyr Gly Pro Leu Thr Ile Arg Arg Val Leu Trp Ala | |
| | 35 40 45 | |
| 35 | GTG GCC TTC GTG GGC TCT CTG GGC CTG CTG CTG GTG GAG AGC TCT GAG | 313 |
| | Val Ala Phe Val Gly Ser Leu Gly Leu Leu Leu Val Glu Ser Ser Glu | |
| | 50 55 60 | |
| 40 | AGG GTG TCC TAC TAC TTC TCC TAC CAG CAT GTC ACT AAG GTG GAC GAA | 361 |
| | Arg Val Ser Tyr Tyr Phe Ser Tyr Gln His Val Thr Lys Val Asp Glu | |
| | 65 70 75 | |
| 45 | GTG GTG GCT CAA AGC CTG GTC TTC CCA GCT GTG ACC CTC TGT AAC CTC | 409 |
| | Val Val Ala Gln Ser Leu Val Phe Pro Ala Val Thr Leu Cys Asn Leu | |
| | 80 85 90 | |
| 50 | AAT GGC TTC CGG TTC TCC AGG CTC ACC ACC AAC GAC CTG TAC CAT GCT | 457 |
| | Asn Gly Phe Arg Phe Ser Arg Leu Thr Thr Asn Asp Leu Tyr His Ala | |
| | 95 100 105 110 | |
| 55 | GGG GAG CTG CTG GCC CTG CTG GAT GTC AAC CTG CAG ATC CCG GAC CCC | 505 |
| | Gly Glu Leu Leu Ala Leu Leu Asp Val Asn Leu Gln Ile Pro Asp Pro | |
| | 115 120 125 | |
| 55 | CAT CTG GCT GAC CCC TCC GTG CTG GAG GCC CTG CGG CAG AAG GCC AAC | 553 |
| | His Leu Ala Asp Pro Ser Val Leu Glu Ala Leu Arg Gln Lys Ala Asn | |
| | 130 135 140 | |

	TTC AAG CAC TAC AAA CCC AAG CAG TTC AGC ATG CTG GAG TTC CTG CAC	601
	Phe Lys His Tyr Lys Pro Lys Gln Phe Ser Met Leu Glu Phe Leu His	
	145 150 155	
5	CGT GTG GGC CAT GAC CTG AAG GAT ATG ATG CTC TAC TGC AAG TTC AAA	649
	Arg Val Gly His Asp Leu Lys Asp Met Met Leu Tyr Cys Lys Phe Lys	
	160 165 170	
10	GGG CAG GAG TGC GGC CAC CAA GAC TTC ACC ACA GTG TTT ACA AAA TAT	697
	Gly Gln Glu Cys Gly His Gln Asp Phe Thr Thr Val Phe Thr Lys Tyr	
	175 180 185 190	
15	GGG AAG TGT TAC ATG TTT AAC TCA GGC GAG GAT GGC AAA CCT CTG CTC	745
	Gly Lys Cys Tyr Met Phe Asn Ser Gly Glu Asp Gly Lys Pro Leu Leu	
	195 200 205	
20	ACC ACG GTC AAG GGG GGG ACA GGC AAC GGG CTG GAG ATC ATG CTG GAC	793
	Thr Thr Val Lys Gly Gly Thr Gly Asn Gly Leu Glu Ile Met Leu Asp	
	210 215 220	
25	ATT CAG CAG GAT GAG TAC CTG CCC ATC TGG GGA GAG ACA GAG GAA ACG	841
	Ile Gln Gln Asp Glu Tyr Leu Pro Ile Trp Gly Glu Thr Glu Glu Thr	
	225 230 235	
30	ACA TTT GAA GCA GGA GTG AAA GTT CAG ATC CAC AGT CAG TCT GAG CCA	889
	Thr Phe Glu Ala Gly Val Lys Val Gln Ile His Ser Gln Ser Glu Pro	
	240 245 250	
35	CCT TTC ATC CAA GAG CTG GGC TTT GGG GTG GCT CCA GGG TTC CAG ACC	937
	Pro Phe Ile Gln Glu Leu Gly Phe Gly Val Ala Pro Gly Phe Gln Thr	
	255 260 265 270	
40	TTT GTG GCC ACA CAG GAG CAG AGG CTC ACA TAC CTG CCC CCA CCG TGG	985
	Phe Val Ala Thr Gln Glu Gln Arg Leu Thr Tyr Leu Pro Pro Pro Trp	
	275 280 285	
45	GGT GAG TGC CGA TCC TCA GAG ATG GGC CTC GAC TTT TTT CCT GTT TAC	1033
	Gly Glu Cys Arg Ser Ser Glu Met Gly Leu Asp Phe Phe Pro Val Tyr	
	290 295 300	
50	AGC ATC ACC GCC TGT AGG ATT GAC TGT GAG ACC CGC TAC ATT GTG GAA	1081
	Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Ile Val Glu	
	305 310 315	
55	AAC TGC AAC TGC CGC ATG GTT CAC ATG CCA GGG GAT GCC CCT TTT TGT	1129
	Asn Cys Asn Cys Arg Met Val His Met Pro Gly Asp Ala Pro Phe Cys	
	320 325 330	
60	ACC CCT GAG CAG CAC AAG GAG TGT GCA GAG CCT GCC CTA GGT CTG TTG	1177
	Thr Pro Glu Gln His Lys Glu Cys Ala Glu Pro Ala Leu Gly Leu Leu	
	335 340 345 350	
65	GCG GAA AAG GAC AGC AAT TAC TGT CTC TGC AGG ACA CCC TGC AAC CTA	1225
	Ala Glu Lys Asp Ser Asn Tyr Cys Leu Cys Arg Thr Pro Cys Asn Leu	
	355 360 365	
70	ACC CGC TAC AAC AAA GAG CTC TCC ATG GTG AAG ATC CCC AGC AAG ACA	1273
	Thr Arg Tyr Asn Lys Glu Leu Ser Met Val Lys Ile Pro Ser Lys Thr	
	370 375 380	

	TCA GCC AAG TAC CTT GAG AAG AAA TTT AAC AAA TCA GAA AAA TAT ATC	1321
	Ser Ala Lys Tyr Leu Glu Lys Lys Phe Asn Lys Ser Glu Lys Tyr Ile	
	385 390 395	
5	TCA GAG AAC ATC CTT GTT CTG GAT ATA TTT TTT GAA GCT CTC AAT TAT	1369
	Ser Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Ala Leu Asn Tyr	
	400 405 410	
10	GAG ACA ATT GAA CAG AAG AAG GCG TAT GAA GTT GCT GCC TTA CTT GGT	1417
	Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Val Ala Ala Leu Leu Gly	
	415 420 425 430	
15	GAT ATT GGT GGT CAG ATG GGA TTG TTC ATT GGT GCT AGT ATC CTT ACA	1465
	Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu Thr	
	435 440 445	
20	ATA CTA GAG CTC TTT GAT TAT ATT TAT GAG CTG ATC AAA GAG AAG CTA	1513
	Ile Leu Glu Leu Phe Asp Tyr Ile Tyr Glu Leu Ile Lys Glu Lys Leu	
	450 455 460	
25	TTA GAC CTG CTT GGC AAA GAG GAG GAT GAA GGG AGC CAC GAT GAG AAT	1561
	Leu Asp Leu Leu Gly Lys Glu Glu Asp Glu Gly Ser His Asp Glu Asn	
	465 470 475	
30	GTG AGT ACT TGT GAC ACA ATG CCA AAC CAC TCT GAA ACC ATC AGT CAC	1609
	Val Ser Thr Cys Asp Thr Met Pro Asn His Ser Glu Thr Ile Ser His	
	480 485 490	
35	ACT GTG AAC GTG CCC CTG CAG ACG ACC CTG GGG ACC CTG GAA GAA ATA	1657
	Thr Val Asn Val Pro Leu Gln Thr Thr Leu Gly Thr Leu Glu Glu Ile	
	495 500 505 510	
	GCC TGC TGA	1666
	Ala Cys *	
	512	

INFORMATION CONCERNANT LA SEQ ID NO:4 :

- i) CARACTERISTIQUE DE LA SEQUENCE :
- 5 A) LONGUEUR : 3647 paires de base
- B) TYPE : acide nucléique
- C) NOMBRE DE BRINS : double
- D) CONFIGURATION : linéaire
- ii) TYPE DE MOLECULE : ADN
- 10 vi) ORIGINE : rat
- ix) CARACTERISTIQUE
- A) NOM/CLE : ASIC1B
- B) LOCALISATION : 109 .. 1785
- 15 xi) DESCRIPTION DE LA SEQUENCE : SEQ ID NO:4 :

```

CTGCCACAGA GGCTCTGGTG AGGAAGGACA GACAGCTGGA CCGGCGCAGA CCTAGCCGAA      60
20 GTCCAACCTC CGTCCCTTCT GGTGGCTTCT TCCTGTCTCC TGAACAAG ATG CCC ATC      117
                               Met Pro Ile
                               1      3

CAG ATC TTT TGT TCT GTG TCA TTC TCC TCT GGA GAG GAG GCC CCG GGA      165
25 Gln Ile Phe Cys Ser Val Ser Phe Ser Ser Gly Glu Glu Ala Pro Gly
   5              10              15

TCC ATG GCA GAT ATC TGG GGT CCC CAC CAC CAC CGG CAG CAG CAG GAC      213
30 Ser Met Ala Asp Ile Trp Gly Pro His His His Arg Gln Gln Gln Asp
   20              25              30              35

AGC TCA GAA TCG GAA GAA GAG GAA GAG AAG GAA ATG GAG GCA GGG TCG      261
   Ser Ser Glu Ser      40 Glu Glu Glu Glu Glu Lys Glu Met Glu Ala Gly Ser
                               45              50

GAG TTG GAT GAG GGT GAT GAC TCA CCT AGG GAC TTG GTG GCC TTC GCC      309
35 Glu Leu Asp Glu Gly Asp Asp Ser Pro Arg Asp Leu Val Ala Phe Ala
   55              60              65

AAC AGC TGT ACC TTC CAT GGT GCC AGC CAT GTG TTT GTG GAA GGG GGC      357
40 Asn Ser Cys Thr Phe His Gly Ala Ser His Val Phe Val Glu Gly Gly
   70              75              80

CCA GGG CCA AGG CAG GCC TTA TGG GCA GTG GCC TTT GTC ATA GCA CTG      405
45 Pro Gly Pro Arg Gln Ala Leu Trp Ala Val Ala Phe Val Ile Ala Leu
   85              90              95

GGT GCC TTC CTG TGC CAG GTA GGG GAC CGC GTT GCT TAT TAC CTC AGC      453
50 Gly Ala Phe Leu Cys Gln Val Gly Asp Arg Val Ala Tyr Tyr Leu Ser
   100              105              110              115

TAC CCA CAC GTG ACT TTG CTA GAC GAA GTG GCC ACC ACG GAG CTG GTC      501
   Tyr Pro His Val Thr Leu Leu Asp Glu Val Ala Thr Thr Glu Leu Val
   120              125              130
55

```

	TTC CCA GCT GTC ACC TTC TGC AAC ACC AAT GCC GTG CGG TTG TCC CAG	549
	Phe Pro Ala Val Thr Phe Cys Asn Thr Asn Ala Val Arg Leu Ser Gln	
	135 140 145	
5	CTC AGC TAC CCT GAC TTG CTC TAC CTG GCC CCC ATG CTA GGA CTG GAT	597
	Leu Ser Tyr Pro Asp Leu Leu Tyr Leu Ala Pro Met Leu Gly Leu Asp	
	150 155 160	
10	GAG AGT GAT GAC CCC GGG GTG CCC CTT GCT CCT CCT GGC CCA GAG GCT	645
	Glu Ser Asp Asp Pro Gly Val Pro Leu Ala Pro Pro Gly Pro Glu Ala	
	165 170 175	
15	TTC TCC GGG GAG CCT TTT AAC CTC CAT CGT TTC TAT AAT CGC TCT TGC	693
	Phe Ser Gly Glu Pro Phe Asn Leu His Arg Phe Tyr Asn Arg Ser Cys	
	180 185 190 195	
20	CAC CGG CTG GAG GAC ATG CTG CTC TAT TGT TCC TAC TGT GGG GGC CCC	741
	His Arg Leu Glu Asp Met Leu Leu Tyr Cys Ser Tyr Cys Gly Gly Pro	
	200 205 210	
25	TGT GGT CCC CAC AAC TTC TCA GTG GTC TTC ACT CGG TAT GGG AAG TGT	789
	Cys Gly Pro His Asn Phe Ser Val Phe Thr Arg Tyr Gly Lys Cys	
	215 220 225	
30	TAC ACA TTC AAC TCG GGC CAA GAT GGG CGG CCA CGG CTG AAG ACC ATG	837
	Tyr Thr Phe Asn Ser Gly Gln Asp Gly Arg Pro Arg Leu Lys Thr Met	
	230 235 240	
35	AAA GGT GGG ACT GGC AAT GGC CTG GAG ATC ATG CTG GAC ATT CAG CAA	885
	Lys Gly Gly Thr Gly Asn Gly Leu Glu Ile Met Leu Asp Ile Gln Gln	
	245 250 255	
40	GAT GAA TAT TTG CCT GTG TGG GGA GAG ACC GAC GAG ACA TCC TTC GAA	933
	Asp Glu Tyr Leu Pro Val Trp Gly Glu Thr Asp Glu Thr Ser Phe Glu	
	260 265 270 275	
45	GCA GGC ATC AAA GTG CAG ATC CAC AGT CAG GAT GAA CCC CCT TTC ATC	981
	Ala Gly Ile Lys Val Gln Ile His Ser Gln Asp Glu Pro Pro Phe Ile	
	280 285 290	
50	GAC CAG CTG GGC TTT GGT GTG GCT CCA GGT TTC CAG ACG TTT GTG TCT	1029
	Asp Gln Leu Gly Phe Gly Val Ala Pro Gly Phe Gln Thr Phe Val Ser	
	295 300 305	
55	TGC CAG GAG CAG AGG CTC ATC TAC CTG CCC TCA CCC TGG GGC ACC TGC	1077
	Cys Gln Glu Gln Arg Leu Ile Tyr Leu Pro Ser Pro Trp Gly Thr Cys	
	310 315 320	
60	AAT GCT GTT ACC ATG GAC TCG GAT TTC TTC GAC TCC TAC AGC ATC ACT	1125
	Asn Ala Val Thr Met Asp Ser Asp Phe Phe Asp Ser Tyr Ser Ile Thr	
	325 330 335	
65	GCC TGC CGG ATT GAT TGC GAG ACG CGT TAC CTG GTG GAG AAC TGC AAC	1173
	Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Leu Val Glu Asn Cys Asn	
	340 345 350 355	

	TGC CGT ATG GTG CAC ATG CCA GGG GAC GCC CCA TAC TGC ACT CCA GAG	1221
	Cys Arg Met Val His Met Pro Gly Asp Ala Pro Tyr Cys Thr Pro Glu	
	360 365 370	
5	CAG TAC AAG GAG TGT GCA GAT CCT GCC CTG GAC TTC CTA GTG GAG AAA	1269
	Gln Tyr Lys Glu Cys Ala Asp Pro Ala Leu Asp Phe Leu Val Glu Lys	
	375 380 385	
10	GAC CAG GAA TAC TGC GTG TGT GAG ATG CCT TGC AAC CTG ACC CGC TAC	1317
	Asp Gln Glu Tyr Cys Val Cys Glu Met Pro Cys Asn Leu Thr Arg Tyr	
	390 395 400	
15	GGC AAG GAG CTG TCC ATG GTC AAG ATC CCA AGC AAA GCC TCC GCC AAG	1365
	Gly Lys Glu Leu Ser Met Val Lys Ile Pro Ser Lys Ala Ser Ala Lys	
	405 410 415	
20	TAC CTG GCC AAG AAG TTC AAC AAA TCG GAG CAG TAC ATA GGG GAG AAC	1413
	Tyr Leu Ala Lys Lys Phe Asn Lys Ser Glu Gln Tyr Ile Gly Glu Asn	
	420 425 430 435	
25	ATT CTG GTG CTG GAC ATT TTC TTT GAA GTC CTC AAC TAT GAG ACC ATC	1461
	Ile Leu Val Leu Asp Ile Phe Phe Glu Val Leu Asn Tyr Glu Thr Ile	
	440 445 450	
30	GAG CAG AAA AAG GCC TAT GAG ATC GCA GGG CTG TTG GGT GAC ATC GGG	1509
	Glu Gln Lys Lys Ala Tyr Glu Ile Ala Gly Leu Leu Gly Asp Ile Gly	
	455 460 465	
35	GGC CAG ATG GGG TTG TTC ATC GGT GCC AGC ATC CTC ACC GTG CTG GAA	1557
	Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu Thr Val Leu Glu	
	470 475 480	
40	CTC TTT GAC TAT GCC TAC GAG GTC ATT AAG CAC AGG CTG TGC AGA CGT	1605
	Leu Phe Asp Tyr Ala Tyr Glu Val Ile Lys His Arg Leu Cys Arg Arg	
	485 490 495	
45	GGA AAG TGC CAG AAG GAG GCT AAG AGG AGC AGC GCA GAC AAG GGC GTG	1653
	Gly Lys Cys Gln Lys Glu Ala Lys Arg Ser Ser Ala Asp Lys Gly Val	
	500 505 510 515	
50	GCG CTC AGC CTG GAT GAC GTC AAA AGA CAC AAT CCC TGC GAG AGC CTC	1701
	Ala Leu Ser Leu Asp Asp Val Lys Arg His Asn Pro Cys Glu Ser Leu	
	520 525 530	
55	CGA GGA CAT CCT GCC GGG ATG ACG TAC GCT GCC AAC ATC CTA CCT CAC	1749
	Arg Gly His Pro Ala Gly Met Thr Tyr Ala Ala Asn Ile Leu Pro His	
	535 540 545	
60	CAT CCC GCT CGA GGC ACG TTT GAG GAC TTT ACC TGC TAA GCCCTCGCAG	1798
	His Pro Ala Arg Gly Thr Phe Glu Asp Phe Thr Cys *	
	550 55 559	
	GCCGCTGTAC CAAAGGCCTA GGTGGGGAGG GCTGGGGGAG CAAGGGGCCC CCAACTGCCC	1858
65	CCAGCTACCC TGTGGACTTA ACTGCATTCC TGGTCAGTGG TTCCCTCTTG TCTGTGGTGA	1918
	GAAAGGAGTC TTGACCATAG AGTCCTCTCC CAGCCTCTAT CCCATCTTTT TATTTTAATT	1978
	TAATCACATT TGCTCTGTAA TATTGCTTGA GGCTGGGGAT CGTGATTTC CCCCAGTTCT	2038

	TTTATTGTTG AGAATAGTTT TCTCTATTCT GGGTTTTCTG TTATTTCAAA TGAATCTGCA	2098
	AATTGCTCTT CCCATCTCTA TGAAGAATTG CGTTGGAATT TTGATGGGGA TTGTATTGAA	2158
5	TCTGTAGATT GCCTTTGGTA AGATGGCCAT TTTTACTATG TTAATCCTGC CAATTCATGA	2218
	GCAAGGGAGA TCTTTCTATC TCTGAAATCT ACTTCAGTTT CTTTCTTCAG AGACTTGAAG	2278
10	TTCTTGTCAT AAAAATCTTT TTGGTTAGAG CCACACCAAG GTATTTTATA TTGTTTGTA	2338
	CTATTGTGAA TGGTGTCAAT TCCCTAATTT CCTTCTCAGC CTAATTATCC TTTGAGTAGA	2398
	GGAAGGCTTC TGATTTGTTT GGGTTAATTT TATACCCAGC TGCTTTGCTA AAGTTCTTTA	2458
15	TCAGGTTTAG GTGTCTCTG GTGGAATTT TGGGGTCACG TAAGAATACT ATTATATCAT	2518
	CTGCAATAG TGATATTTCA CTTCTTCCTT TCCAATTTCT ATCCCTCTGG GGACTTTTTG	2578
20	TTGTCTAATT GCTCTGGCTA GGACTTCAAA TTCTATATTG AATAGATAGG GAGAGAGTGG	2638
	GCAGCCTTGT CTAGTTCCTG GTTTTCGTGG GATCGCTTCA AATTTCTCTC CATTTAGTTT	2698
	GATATTGGCT ACTGGTTTGC TGTATATGGC TTTTACTGTA CTTAGGTATG GGCCTTGAAT	2758
25	TCCTGATATT TCCAAGACTT TTAACATGAA GGGGTTTTGA AATTTGCCAA ATGCTTTCTC	2818
	AGCATCTAAT GAGATGATCA TGTGCCCTCC CCCCACCTTG AGTTTGTTTA TATAGTGGGT	2878
30	TACATGAAAG GATCATTCTT AATAGTCCAC AAGTCTGCCA AATCTTGCTG ATTGTGACTC	2938
	ATTTCCATAG CAGGCTCTAT AACTTCTCTA ACAGATTGCA TTAAACTCTG CTTGGGGAAG	2998
	GCATTACCTC TTGGTTGAAG CAATGTTGTA GTTTCTATGC CTGCTGAGTA AATAGCCTCA	3058
35	AGTCCAAGTA CTGCCCAGA CTAATGATCA AACGTATCCA GGAGTTCAT ACCAGAGATG	3118
	TACTCTTCTC TCCTTTGAAG TACATTGCTG GAAGAGTAAT TGTGTTTGCT AGAGATACTC	3178
40	CTTCGAACTG CAAAAGAAAT CTCTTGGCTA AGCATATAAT CAAGCCTCAG GTTTTCTTTT	3238
	TATTAAATAG CTGCTTGTA GAAAGTGGAC ACTAAGCATA TACCTCAAAG GGAGACAGAA	3298
	TGACTCTGTG CCTTCACTGA TGGAAAGTCTG GGTTACAAAT TACATCAGAA GAACCTATCA	3358
45	TAGTGAAACA TCTCATTCCC CTGGTATAAT CCCTTCTAGA AATACACTTG TGACTCTGAA	3418
	ATGTTATAAT CGTGACAACT AGGCTGTTAC AGATACACCA AGTTAAATTT GATAGAGAAA	3478
50	CCAGGCTTGG AGCCTCATGT CCATAGGGCA AGAGGAAGAT GCTGAGTGTT TAAGGTTGGT	3538
	TTGAGCGAAG AACAATACCT TGTGTCACAA AAATGAAAGG AAAAAAGAAA AAAGGAAAGA	3598
	AGGAAAGAAA GAGAGAGAAA GAAAAAGAAA GAAAGAAAAA AAAAAAAA	3647

INFORMATION CONCERNANT LA SEQ ID NO:5 :

- i) CARACTERISTIQUE DE LA SEQUENCE :
- 5 A) LONGUEUR 1602 paires de base
- B) TYPE : acide nucléique
- C) NOMBRE DE BRINS : double
- D) CONFIGURATION : linéaire
- ii) TYPE DE MOLECULE : ADN
- 10 vi) ORIGINE : rat
- ix) CARACTERISTIQUE
- A) NOM/CLE : DRASIC
- B) LOCALISATION : 1 .. 1602
- 15 xi) DESCRIPTION DE LA SEQUENCE : SEQ ID NO:5 :
- | | | |
|----|--|-----|
| 20 | ATG AAA CCT CGC TCC GGA CTG GAG GAG GCC CAG CGG CGA CAG GCC TCA
Met Lys Pro Arg Ser Gly Leu Glu Glu Ala Gln Arg Arg Gln Ala Ser | 48 |
| | 1 5 10 15 | |
| | GAC ATC CGG GTG TTT GCC AGC AGC TGC ACA ATG CAT GGT CTG GGC CAC
Asp Ile Arg Val Phe Ala Ser Ser Cys Thr Met His Gly Leu Gly His | 96 |
| 25 | 20 25 30 | |
| | ATC TTT GGC CCT GGA GGC CTG ACC CTG CGC CGA GGG CTG TGG GCC ACA
Ile Phe Gly Pro Gly Gly Leu Thr Leu Arg Arg Gly Leu Trp Ala Thr | 144 |
| | 35 40 45 | |
| 30 | GCT GTG CTC CTG TCG CTG GCG GCC TTC CTC TAC CAG GTG GCT GAG CGG
Ala Val Leu Leu Ser Leu Ala Ala Phe Leu Tyr Gln Val Ala Glu Arg | 192 |
| | 50 55 60 | |
| 35 | GTT CGC TAC TAT GGG GAG TTC CAC CAT AAG ACC ACC CTG GAT GAG CGT
Val Arg Tyr Tyr Gly Glu Phe His His Lys Thr Thr Leu Asp Glu Arg | 240 |
| | 65 70 75 80 | |
| 40 | GAG AGC CAC CAG CTC ACC TTC CCA GCT GTG ACT CTG TGT AAT ATC AAC
Glu Ser His Gln Leu Thr Phe Pro Ala Val Thr Leu Cys Asn Ile Asn | 288 |
| | 85 90 95 | |
| 45 | CCA CTG CGC CGC TCA CGC CTC ACA CCC AAT GAC TTG CAC TGG GCT GGA
Pro Leu Arg Arg Ser Arg Leu Thr Pro Asn Asp Leu His Trp Ala Gly | 336 |
| | 100 105 110 | |
| | ACA GCG CTG CTG GGC CTG GAC CCT GCT GAA CAT GCT GCC TAC CTT CGT
Thr Ala Leu Leu Gly Leu Asp Pro Ala Glu His Ala Ala Tyr Leu Arg | 384 |
| | 115 120 125 | |
| 50 | GCA CTG GGC CAG CCC CCC GCA CCA CCT GGC TTC ATG CCC AGT CCG ACC
Ala Leu Gly Gln Pro Pro Ala Pro Pro Gly Phe Met Pro Ser Pro Thr | 432 |
| | 130 135 140 | |
| 55 | TTT GAC ATG GCA CAA CTC TAC GCC AGA GCC GGC CAC TCC CTT GAG GAC
Phe Asp Met Ala Gln Leu Tyr Ala Arg Ala Gly His Ser Leu Glu Asp | 480 |
| | 145 150 155 160 | |

	ATG TTG TTG GAT TGC CGA TAC CGT GGC CAG CCC TGT GGG CCT GAG AAC	528
	Met Leu Leu Asp Cys Arg Tyr Arg Gly Gln Pro Cys Gly Pro Glu Asn	
	165 170 175	
5	TTC ACA GTG ATC TTT ACT CGA ATG GGG CAA TGC TAC ACC TTC AAC TCT	576
	Phe Thr Val Ile Phe Thr Arg Met Gly Gln Cys Tyr Thr Phe Asn Ser	
	180 185 190	
10	GGT GCC CAC GGT GCA GAG CTG CTC ACC ACT CCA AAG GGT GGT GCT GGC	624
	Gly Ala His Gly Ala Glu Leu Thr Thr Pro Lys Gly Gly Ala Gly	
	195 200 205	
15	AAC GGA CTG GAG ATT ATG CTA GAT GTA CAG CAA GAG GAG TAT CTG CCC	672
	Asn Gly Leu Glu Ile Met Leu Asp Val Gln Gln Glu Glu Tyr Leu Pro	
	210 215 220	
20	ATC TGG AAG GAC ATG GAA GAG ACC CCG TTT GAG GTG GGG ATC CGA GTG	720
	Ile Trp Lys Asp Met Glu Glu Thr Pro Phe Glu Val Gly Ile Arg Val	
	225 230 235 240	
25	CAG ATT CAC AGC CAG GAT GAG CCC CCT GCC ATT GAC CAG CTG GGC TTC	768
	Gln Ile His Ser Gln Asp Glu Pro Pro Ala Ile Asp Gln Leu Gly Phe	
	245 250 255	
30	GGG GCA GCC CCA GGC CAT CAG ACT TTT GTG TCC TGT CAG CAG CAG CAA	816
	Gly Ala Ala Pro Gly His Gln Thr Phe Val Ser Cys Gln Gln Gln	
	260 265 270	
35	CTG AGT TTC CTG CCA CCA CCC TGG GGT GAC TGC AAT ACC GCA TCT TTG	864
	Leu Ser Phe Leu Pro Pro Pro Trp Gly Asp Cys Asn Thr Ala Ser Leu	
	275 280 285	
40	GAT CCC GAC GAC TTT GAT CCA GAG CCC TCT GAT CCC TTG GGT TCC CCC	912
	Asp Pro Asp Asp Phe Asp Pro Glu Pro Ser Asp Pro Leu Gly Ser Pro	
	290 295 300	
45	AGA CCC AGA CCC AGC CCT CCT TAT AGT TTA ATA GGT TGT CGC CTG GCC	960
	Arg Pro Arg Pro Ser Pro Pro Tyr Ser Leu Ile Gly Cys Arg Leu Ala	
	305 310 315 320	
50	TGT GAG TCT CGC TAT GTG GCT CGG AAG TGT GGC TGT CGA ATG ATG CAT	1008
	Cys Glu Ser Arg Tyr Val Ala Arg Lys Cys Gly Cys Arg Met Met His	
	325 330 335	
55	ATG CCT GGA AAC TCC CCA GTG TGC AGC CCC CAG CAG TAC AAG GAC TGC	1056
	Met Pro Gly Asn Ser Pro Val Cys Ser Pro Gln Gln Tyr Lys Asp Cys	
	340 345 350	
60	GCC AGC CCA GCT CTG GAC GCT ATG CTG CGA AAG GAC ACG TGT GTC TGC	1104
	Ala Ser Pro Ala Leu Asp Ala Met Leu Arg Lys Asp Thr Cys Val Cys	
	355 360 365	
65	CCC AAC CCG TGC GCT ACT ACA CGC TAT GCC AAG GAG CTC TCC ATG GTG	1152
	Pro Asn Pro Cys Ala Thr Thr Arg Tyr Ala Lys Glu Leu Ser Met Val	
	370 375 380	
70	CGG ATT CCC AGC CGC GCG TCA GCT CGC TAC CTG GCC CGG AAA TAC AAC	1200
	Arg Ile Pro Ser Arg Ala Ser Ala Arg Tyr Leu Ala Arg Lys Tyr Asn	
	385 390 395 400	

	CGC AGC GAG TCC TAC ATT ACG GAG AAT GTA CTG GTT CTG GAT ATC TTC	1248
	Arg Ser Glu Ser Tyr Ile Thr Glu Asn Val Leu Val Leu Asp Ile Phe	
	405 410 415	
5	TTT GAG GCC CTC AAC TAT GAA GCG GTG GAA CAA AAG GCG GCC TAT GAA	1296
	Phe Glu Ala Leu Asn Tyr Glu Ala Val Glu Gln Lys Ala Ala Tyr Glu	
	420 425 430	
10	GTG TCG GAG CTG CTG GGA GAC ATT GGG GGA CAG ATG GGA CTG TTT ATT	1344
	Val Ser Glu Leu Leu Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile	
	435 440 445	
15	GGA GCA AGC CTG CTT ACC ATC CTT GAG ATC CTC GAC TAT CTC TGT GAG	1392
	Gly Ala Ser Leu Leu Thr Ile Leu Glu Ile Leu Asp Tyr Leu Cys Glu	
	450 455 460	
20	GTT TTC CAA GAC AGA GTC CTG GGG TAT TTC TGG AAC AGA AGG AGC GCT	1440
	Val Phe Gln Asp Arg Val Leu Gly Tyr Phe Trp Asn Arg Arg Ser Ala	
	465 470 475 480	
25	CAA AAG CGC TCT GGC AAC ACT CTG CTC CAG GAA GAG TTG AAT GGC CAT	1488
	Gln Lys Arg Ser Gly Asn Thr Leu Leu Gln Glu Glu Leu Asn Gly His	
	485 490 495	
30	CGA ACA CAT GTT CCC CAC CTC AGC CTA GGG CCC AGG CCT CCT ACC ACT	1536
	Arg Thr His Val Pro His Leu Ser Leu Gly Pro Arg Pro Pro Thr Thr	
	500 505 510	
35	CCC TGT GCT GTC ACC AAG ACA CTC TCT GCC TCC CAC CGT ACC TGT TAC	1584
	Pro Cys Ala Val Thr Lys Thr Leu Ser Ala Ser His Arg Thr Cys Tyr	
	515 520 525	
	CTC GTC ACA AGG CTC TAG	1602
	Leu Val Thr Arg Leu *	
	530 533	

INFORMATION CONCERNANT LA SEQ ID NO:6 :

i) CARACTERISTIQUE DE LA SEQUENCE :

5 A) LONGUEUR 1948 paires de base

B) TYPE : acide nucléique

C) NOMBRE DE BRINS : double

D) CONFIGURATION : linéaire

ii) TYPE DE MOLECULE : ADN

10 vi) ORIGINE : rat

ix) CARACTERISTIQUE

A) NOM/CLE : MDEG2

B) LOCALISATION : 16 .. 1707

15 xi) DESCRIPTION DE LA SEQUENCE : SEQ ID NO:6 :

	CCTCGGGCTG AATGA ATG AGC CGG AGC GGC GGA GCC CGG CTG CCC GCG ACC	51
	Met Ser Arg Ser Gly Gly Ala Arg Leu Pro Ala Thr	
20	1 5 10	
	GCG CTC AGC GGC CCG GGA CGC TTC CGT ATG GCC CGC GAG CAG CCG GCG	99
	Ala Leu Ser Gly Pro Gly Arg Phe Arg Met Ala Arg Glu Gln Pro Ala	
	15 20 25	
25	CCC GTG GCG GTG GCG GCA GCT AGG CAG CCC GGA GGA GAC CGG AGC GGC	147
	Pro Val Ala Val Ala Ala Ala Arg Gln Pro Gly Gly Asp Arg Ser Gly	
	30 35 40	
30	GAT CCG GCG CTG CAG GGG CCA GGG GTC GCC CGC AGG GGG CGG CCG TCC	195
	Asp Pro Ala Leu Gln Gly Pro Gly Val Ala Arg Arg Gly Arg Pro Ser	
	45 50 55 60	
35	CTG AGT CGC ACT AAA TTG CAC GGG CTG CGG CAC ATG TGC GCG GGG CGC	243
	Leu Ser Arg Thr Lys Leu His Gly Leu Arg His Met Cys Ala Gly Arg	
	65 70 75	
	ACG GCG GCG GGA GGC TCT TTC CAG CGA CGG GCG CTG TGG GTG CTG GCC	291
40	Thr Ala Ala Gly Gly Ser Phe Gln Arg Arg Ala Leu Trp Val Leu Ala	
	80 85 90	
	TTC TGC ACG TCC CTC GGC TTG CTG CTG TCC TGG TCC TCG AAC CGC CTG	339
	Phe Cys Thr Ser Leu Gly Leu Leu Leu Ser Trp Ser Ser Asn Arg Leu	
	95 100 105	
45	CTC TAC TGG CTC AGC TTC CCG TCA CAC ACA CGA GTG CAC CGT GAG TGG	387
	Leu Tyr Trp Leu Ser Phe Pro Ser His Thr Arg Val His Arg Glu Trp	
	110 115 120	
50	AGC CGC CAG CTG CCG TTC CCC GCC GTC ACC GTG TGC AAC AAC AAC CCC	435
	Ser Arg Gln Leu Pro Phe Pro Ala Val Thr Val Cys Asn Asn Asn Pro	
	125 130 135 140	
55	CTG CGC TTC CCG CGC CTC TCC AAG GGG GAC CTC TAC TAC GCG GGC CAC	483
	Leu Arg Phe Pro Arg Leu Ser Lys Gly Asp Leu Tyr Tyr Ala Gly His	
	145 150 155	

	TGG CTA GGG CTG CTG CTT CCC AAC CGC ACC GCG CGC CCG CTG GTC AGC	531
	Trp Leu Gly Leu Leu Leu Pro Asn Arg Thr Ala Arg Pro Leu Val Ser	
	160 165 170	
5	GAG CTG CTG CGG GGC GAC GAG CCG CGC CGC CAG TGG TTC CGC AAA CTG	579
	Glu Leu Leu Arg Gly Asp Glu Pro Arg Arg Gln Trp Phe Arg Lys Leu	
	175 180 185	
10	GCC GAC TTC CGC CTC TTC CTG CCG CCG CGC CAC TTC GAG GGC ATC AGC	627
	Ala Asp Phe Arg Leu Phe Leu Pro Pro Arg His Phe Glu Gly Ile Ser	
	190 195 200	
15	GCT GCC TTC ATG GAC CGT TTG GGC CAC CAG CTG GAG GAT ATG CTG CTC	675
	Ala Ala Phe Met Asp Arg Leu Gly His Gln Leu Glu Asp Met Leu Leu	
	205 210 215 220	
20	TCC TGC AAG TAC CGG GGC GAG CTC TGT GGC CCG CAC AAC TTC TCC TCA	723
	Ser Cys Lys Tyr Arg Gly Glu Leu Cys Gly Pro His Asn Phe Ser Ser	
	225 230 235	
25	GTG TTT ACA AAA TAC GGG AAG TGT TAC ATG TTT AAC TCA GGC GAG GAT	771
	Val Phe Thr Lys Tyr Gly Lys Cys Tyr Met Phe Asn Ser Gly Glu Asp	
	240 245 250	
30	GGC AAG CCG CTG CTC ACC ACG GTC AAG GGG GGG ACG GGC AAC GGG CTG	819
	Gly Lys Pro Leu Leu Thr Thr Val Lys Gly Gly Thr Gly Asn Gly Leu	
	255 260 265	
35	GAG ATC ATG CTG GAC ATT CAG CAA GAT GAG TAC CTG CCC ATC TGG GGA	867
	Glu Ile Met Leu Asp Ile Gln Gln Asp Glu Tyr Leu Pro Ile Trp Gly	
	270 275 280	
40	GAG ACA GAG GAA ACA ACG TTT GAA GCA GGA GTG AAG GTT CAG ATC CAC	915
	Glu Thr Glu Glu Thr Thr Phe Glu Ala Gly Val Lys Val Gln Ile His	
	285 290 295 300	
45	AGT CAG TCT GAG CCG CCT TTC ATC CAA GAG CTG GGC TTT GGG GTG GCT	963
	Ser Gln Ser Glu Pro Pro Phe Ile Gln Glu Leu Gly Phe Gly Val Ala	
	305 310 315	
50	CCG GGG TTC CAG ACC TTC GTG GCC ACA CAA GAG CAG AGG CTC ACA TAT	1011
	Pro Gly Phe Gln Thr Phe Val Ala Thr Gln Glu Gln Arg Leu Thr Tyr	
	320 325 330	
55	CTG CCC CCA CCA TGG GGG GAG TGC CCG TCC TCA GAG ATG GGA CTC GAC	1059
	Leu Pro Pro Pro Trp Gly Glu Cys Arg Ser Ser Glu Met Gly Leu Asp	
	335 340 345	
55	TTC TTT CCT GTT TAC AGC ATC ACA GCC TGT CGG ATT GAC TGT GAG ACC	1107
	Phe Phe Pro Val Tyr Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr	
	350 355 360	
55	CGC TAC ATC GTG GAG AAC TGT AAC TGC CGC ATG GTC CAC ATG CCA GGG	1155
	Arg Tyr Ile Val Glu Asn Cys Asn Cys Arg Met Val His Met Pro Gly	
	365 370 375 380	

	GAC GCC CCT TTC TGC ACC CCT GAG CAG CAC AAG GAG TGT GCA GAG CCT	1203
	Asp Ala Pro Phe Cys Thr Pro Glu Gln His Lys Glu Cys Ala Glu Pro	
	385 390 395	
5	GCC CTC GGT CTA CTG GCA GAA AAG GAC AGC AAT TAC TGT CTC TGC AGG	1251
	Ala Leu Gly Leu Leu Ala Glu Lys Asp Ser Asn Tyr Cys Leu Cys Arg	
	400 405 410	
10	ACA CCC TGC AAC CTG ACA CGC TAC AAC AAA GAG CTC TCC ATG GTG AAG	1299
	Thr Pro Cys Asn Leu Thr Arg Tyr Asn Lys Glu Leu Ser Met Val Lys	
	415 420 425	
15	ATC CCC AGC AAG ACG TCA GCC AAG TAC TTA GAG AAG AAA TTT AAC AAA	1347
	Ile Pro Ser Lys Thr Ser Ala Lys Tyr Leu Glu Lys Lys Phe Asn Lys	
	430 435 440	
20	TCG GAA AAA TAT ATC TCA GAG AAC ATT CTT GTT CTG GAC ATA TTT TTT	1395
	Ser Glu Lys Tyr Ile Ser Glu Asn Ile Leu Val Leu Asp Ile Phe Phe	
	445 450 455 460	
25	GAG GCG CTC AAT TAC GAA ACA ATT GAA CAG AAG AAG GCG TAT GAA GTT	1443
	Glu Ala Leu Asn Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Val	
	465 470 475	
30	GCT GCC TTA CTT GGT GAC ATC GGT GGT CAG ATG GGA CTG TTC ATT GGT	1491
	Ala Ala Leu Leu Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly	
	480 485 490	
35	GCT AGT CTC CTC ACA ATA CTA GAG CTC TTT GAT TAT ATT TAT GAG CTG	1539
	Ala Ser Leu Leu Thr Ile Leu Glu Leu Phe Asp Tyr Ile Tyr Glu Leu	
	495 500 505	
40	ATC AAA GAG AAG CTA TTA GAC CTG CTT GGC AAA GAA GAA GAG GAA GGG	1587
	Ile Lys Glu Lys Leu Leu Asp Leu Leu Gly Lys Glu Glu Glu Glu Gly	
	510 515 520	
45	AGC CAC GAT GAG AAC ATG AGC ACC TGT GAC ACA ATG CCA AAC CAC TCT	1635
	Ser His Asp Glu Asn Met Ser Thr Cys Asp Thr Met Pro Asn His Ser	
	525 530 535 540	
50	GAA ACC ATC AGC CAC ACT GTG AAC GTG CCC CTG CAG ACA GCT TTG GGC	1683
	Glu Thr Ile Ser His Thr Val Asn Val Pro Leu Gln Thr Ala Leu Gly	
	545 550 555	
55	ACC CTG GAG GAG ATT GCC TGC TGA CACCTCTCAG GCAACGCAGC ACCTCCAAAC	1737
	Thr Leu Glu Glu Ile Ala Cys *	
	560 563	
60	AGACCTTAAA GGCCCAAGAC CTAGGACAGG AGACAGCAAG CGCAGGTGGG ATCGCCCCTG	1797
	ACGACTGAAA GAAGCAGAGC CCCCATATG CACACATTGC GAACTTCTGC CAAACCTCAC	1857
	CTGGCCACAT CTGACATGAA CCGTCCCGGG CCCTGCGTCA TGTCCCTCGC AGGACCGATG	1917
	AGTCGCACTC CGGAAGTGTG CAAGAACTAA C	1948

SEQ ID No. 7

ACGACGGGGTTCTGGCCATGAAGCCCACCTCAGGCCAGAGGAGGCCCCGGCGGCCAGCCT
CGGACATCCGCGTGTTGCGCCAGCAACTGCTCGATGCACGGGCTGGGCCACGTCTTCGGGC
CAGGCAGCCTGAGCCTGCGCCGGGGGATGTGGGCAGCGCCGTGGTCCTGTCACTGGCCA
CCTTCCTCTACCAGGTGGCTGAGAGGGTGCGCTACTACAGGGAGTTCACCACCAGACTG
CCCTGGATGAGCGAGAAAGCCACCGGCTCATCTCCCGGCTGTACCTGTGCAACATCA
ACCCACTGCGCCGCTCGCGCTAACGCCCAACGACCTGCACTGGGCTGGGTCTGCGCTGC
TGGGCCTGGATCCCGCAGAGCACGCCGCCTTCCTGCGCGCCCTGGGCCGGCCCCCTGCAC
CGCCCGGCTTCATGCCCAGTCCCACCTTTGACATGGCGCAACTCTATGCCCCGTGCTGGGC
ACTCCCTGGATGACATGCTGCTGGACTGTGCTTCCTGGGCCAACCTTGTGGGCCTGAGA
ACTTCACCACGATCTTCACCCGGATGGGAAAGTGCTACACATTTAACTCTGGCGCTGATG
GGGCAGAGCTGCTCACCCTACTAGGGGTGGCATGGGCAATGGGCTGGACATCATGCTGG
ACGTGCAGCAGGAGGAATATCTACCTGTGTGGAGGGACAATGAGGAGACCCGTTTGAGG
TGGGGATCCGAGTGAGATCCACAGCCAGGAGGAGCCGCCCATCATCGATCAGCTGGGCT
TGGGGGTGTCCCGGGCTACCAGACCTTTGTTTCTTGCCAGCAGCAGCAGCTGAGCTTCC
TGCCACCGCCCTGGGGCGATTGCAGTTCAGCATCTCTGAACCCCAACTATGAGCCAGAGC
CCTCTGATCCCCTAGGCTCCCCCAGCCCCAGCCCCAGCCCTCCCTATACCTTTATGGGGT
GTCGCCTGGCCTGCGAAACCCGCTACGTGGCTCGGAAGTGCAGGCTGCCGAATGGTGTACA
TGCCAGGCGAGTGCCAGTGTGCAGCCCCCAGCAGTACAAGAACTGTGCCACCCGGCCA
TAGATGCCATGCTTCGCAAGGACTCGTGCGCTGCCCAACCCGTGCGCCAGCACGCGCT
ACGCCAAGGAGCTCTCATGGTGCGGATCCCGAGCCGCGCCGCGCGCTTCCTGGCCC
GGAAGCTCAACCGCAGCGAGGCCCTACATCGCGGAGAACGTGCTGGCCCTGGACATCTTCT
TTGAGGCCCTCAACTATGAGACCGTGGAGCAGAAGAAGGCCTATGAGATGTCAGAGCTGC
TTGGTGACATTGGGGGCCAGATGGGGCTGTTTCATCGGGGCCAGCCTGCTCACCATCCTCG
AGATCCTAGACTACCTCTGTGAGGTGTTCCGAGACAAGGTCTGGGATATTTCTGGAACC
GACAGCACTCCCAAAGGCACTCCAGCACCAATCTGCTTCAGGAAGGGCTGGGCAGCCATC
GAACCCAAGTTCCCCACCTCAGCCTGGGGCCCAGACCTCCCACCCCTCCCTGTGCCGTCA
CCAAGACTCTCTCCGCTCCACCGCACCTGTACCTTGTACACAGCTCTAGACCTGCT
GTCTGTGTCCTCGGAGCCCCGCCCTGACATCCTGGACATGCCTAGCCTGCACGTAGCTTT
TCCGTCTTCACCCAAATAAAGTCCTAATGCATCAAAAAAAAAAAAAAAAAAAAAA

SEQ ID No. 8

M K P T S G P E E A R R P A S D I R V F A S
N C S M H G L G H V A F G P L S R G R M
W A A V V L S V A T F E L E R R V R Y
Y R E F H V Q L T A D E R L I F P A
V T L C N H I Q N P L R R A A S L A
G S A L L G F M L D P A A F T P L
P A P P G F M L P S P A A Q Q P L
H S L D D M L G D C C N G T F G C A
T T I F T R G M G N E T L D I D V Q Q
L T T P V W R D I L S Q P G Y Q Q E H
Y S Q E P P I Q L S F L G V S G Y Q T F
S V S C Q N Q Q P E R P L P W G D S S A S
V L N P T L Y M G C R L V S A Y V S P C
P Y T V Y M P G D V P A C E T R Q Q P C H
R M V I D M S L R K D S A C P A N A R K
P A K E A M G L R V I A S R A C S A T R
Y A N R S L M S Y Q K A S L A E I G K
L N Y E T L F I G A G Y A L E L L G
Q M G R D Q P V G L G S F L I S Q L G
V F L L Q P P C A V G S H W R T Q V P H
N L P T P C A V G S H W R T Q V P H
P P T L